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Cdh1 overexpression improves emotion and cognitive-related behaviors via regulating hippocampal neuroplasticity in global cerebral ischemia rats

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ABSTRACT

Post-stroke survivors exhibited cognitive deficits and performed emotional impairment. However, the effect of global cerebral ischemia on standard behavioral measures of emotionality and underlying mechanism remain largely unknown. Our previous work identified that down-regulation of Cdh1 contributed to ischemic neuronal death in rat, thus we hypothesized that Cdh1 exerts a role in emotionality after cerebral ischemia, and we investigated the effect of Cdh1 overexpression on neurogenic behaviors and possible mechanisms in transient global cerebral ischemia reperfusion (tGCI/R) rats. A series of behavioral tests were used to evaluate emotion and cognitive related behaviors, and molecular biological techniques were employed to investigate hippocampal neuroplasticity. The results showed that tGCI/R rats displayed anxiety- and depression-like behaviors and a certain degree of cognitive impairment, and these abnormal behaviors accompanied with a loss of hippocampal synapses and dendritic spines, disruption of dendrite arborization and decline in the level of GAP-43, synaptophysin, synapsin and PSD-95. However, Cdh1 overexpression improved negative emotionality, ameliorated cognitive deficits, rescued hippocampal synapses loss, prevented dendritic network disorganization, and increased the level of synaptic-associated proteins after tGCI/R. Taken together, these findings suggest that Cdh1 overexpression exerts a neuroprotective effect by regulating hippocampal neuroplasticity thus improving negative emotionality and cognitive deficits after tGCI/R.

1. Introduction

Ischemic stroke is a major etiology of morbidity and mortality worldwide, accounting for around 85% of all stroke cases with high social and economic burden (Deng et al., 2017). Transient global ischemia occurs in patients who suffer from transient cardiac arrest, severe shock and dysrhythmias or undergo complex cardiac surgery, resulting in a broad range of neurological and emotional dysfunction (Soares et al., 2013). Changes in emotionality represent common features of post-ischemic recovery in humans and the development of post-stroke mood disorders is one symptom of the morbidities that often neglected in stroke victims (Kim, 2016). Meta-analyses of point-prevalence rates suggest that one quarter of stroke-survivors developed post-stroke anxiety and one third manifested post-stroke depression (Campbell Burton et al., 2013; Hackett and Pickles, 2014). Post-stroke emotion and cognitive impairments are some of the most prevalent neuropsychiatric conditions that have been shown to inhibit physical and cognitive recovery and currently requiring therapeutic solutions (Williams et al., 2004).

Ubiquitin-proteasome system (UPS) exerts an important role in the regulation of mood, emotionality and cognitive function (Fuchsberger et al., 2017; Puram and Bonni, 2011). Studies have shown that the expression of UPS-related gene is disturbed in patients with anxiety and depression (Kim et al., 2009; Mouri et al., 2012). The multi-subunit

Abbreviations: APC, anaphase promoting complex; BM, Barnes maze; Cdh1, Cdc20-like protein homologue 1; EPM, elevated plus maze; FST, forced swim test; MWM, morris water maze; NORT, novel object recognition test; NSFT, novelty suppressed feeding test; OFT, open field test; TEM, transmission electron microscope; tGCI/R, transient global cerebral ischemia reperfusion; UPS, ubiquitin proteasome system

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RING finger E3 ubiquitin ligase, anaphase-promoting complex/cyclosome (APC/C) and its co-activator fizzy-related protein homologue/Cdc20-like protein 1 (Cdh1) are important component of the UPS. APC/C-Cdh1 has been reported to be involved in the regulation of neuronal survival, differentiation, axonal growth and synaptic development in central nervous system (Yao et al., 2010). Our previous work (Zhang et al., 2011) indicated that Cdh1 is dominantly expressed in neurons and is significantly downregulated after 15 min of global cerebral ischemia in rat hippocampus, which suggests that Cdh1 may be an important regulatory factor in the process of brain ischemia. Upregulation of Cdh1 can inhibit ischemic neuronal death and affect the ultrastructure of hippocampal synapses (Zhang et al., 2018). However, whether Cdh1 overexpression could reverse negative emotionality induced by tGCI/R is largely unknown.

There is currently no effective drug or therapy available that protects brain from global cerebral ischemia induced neuronal impairment and emotional disorder, and very few studies have examined the effects of global ischemia on emotional behavior in rodents despite the high prevalence of depression and/or anxiety after stroke. In the current study, we aim to investigate the effects of hippocampal Cdh1 overexpression mediated by recombinant lentivirus on anxiety-like, depression-like, and cognitive-related behaviors in tGCI/R rats, aiming to verify the hypothesis that upregulating hippocampal Cdh1 expression level could attenuate ischemia-induced behavioral impairments by regulating hippocampal neuroplasticity, thus to find a new target for stroke therapy.

2. Materials and methods

2.1. Animals and experimental design

Eight-week-old male Sprague-Dawley rats obtained from the Laboratory Animal Center of Huazhong University of Science and Technology, Wuhan, PR China (Certificate No. 42009800002519/SCXK (E)2016-0009) were housed in specific pathogen-free and controlled conditions (environment temperature of 22 ± 2°C, ambient humidity of 50 ± 10%, 12 h light/dark cycle, lights on at 7:00 a.m.) and supplied with standard laboratory chow and water. After one-week acclimatization to the housing facility, rats were randomly assigned to four experimental groups: Sham (n = 16), IR + Saline (n = 30), IR + Lenti-GFP (n = 30) and IR + Lenti-Cdh1-GFP (n = 24), the number of rats in each group was statistically calculated based on our experiences and the detailed experimental protocols are depicted in Fig. 1A.

This study was carried out strictly in adherence to the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised in 1978) and experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Tongji Hospital (Ethics Lot Number: 4952280814/TJ2015A). Numbers of animals used and their sufferings were minimized wherever possible and all rats were daily handled for 2–3 min for four days preceding surgery or behavioral test.

2.2. Lentiviral vector construction and hippocampal stereotaxic microinjection

The construction of recombinant Cdh1 overexpression lentiviral vectors (Lenti-Cdh1-GFP) and normal control vectors (Lenti-GFP) were described previously (Qiu et al., 2013) and commercially amplified by Genechem (Shanghai, China). The vectors contain a CMV-driven green fluorescent protein (GFP) reporter and have been confirmed to successfully upregulate Cdh1 expression level both in vitro and in vivo (Hu et al., 2016; Li et al., 2017b; Lv et al., 2015). Stereotaxic hippocampal virus micro-injection was performed as described in our previous publication (Zhang et al., 2018). Briefly, a mixture of lentivirus (1×10^9 TU/ml) and cationic lipid polybrane (4 μg/ml) were incubated for 30 min at room temperature. Anesthetized rats were fixed to stereotaxic instrument (RWD life-science, Shenzhen, China) with ear bars and incisor clamp, drilling with a cranium drill, the Hamilton brain microinfusion syringe (Hamilton, Nevada, USA) was stereotaxically injected into the hippocampus (coordinates: 3.3 mm caudal to bregma; ± 1.6 mm lateral to midline; 2.8 mm deep from the skull), 2 μl of virus and polybrane mixture was injected into each hippocampus within 4 min, then the syringe was slowly withdrawn and the holes were sealed with bone wax after disinfection with vital iodine. The transfection efficiency was confirmed by RT-PCR and auto GFP fluorescent signal as described in our previous article (Zhang et al., 2018).

2.3. Transient global cerebral ischemia reperfusion model

Two days after hippocampal virus injection, tGCI/R model was performed using the four-vessel occlusion (4-VO) method initially proposed by Pulsinelli and Brierley (1979) and previously used in our lab (Wei et al., 2017; Zhang et al., 2018). Briefly, both vertebral arteries were permanently electrocauterized and bilateral common carotid arteries (CCAs) were isolated on surgical thread under anesthesia (pentobarbital, 45 mg/kg, i.p). After 24 h recovery, the animals were re-anesthetized with light isoflurane inhalation and both CCAs were re-exposed and temporarily occluded with non-traumatic arterial clamps for 10 min. Animals that completely losing their righting reflex within 1 min and showing no convulsive signs were retained for long-term behavioral test. Sham-operated animals underwent the same surgical procedures without arteries occlusion. Core temperature was maintained at 37°C using a feedback-regulated Homoeothermic Blanket Control Unit (Harvard Instruments, Natick, MA) during surgery and recovery period. After surgery, all animals were housed singly for recovery under regular surveillance.

2.4. Body weight and food intake recording

The body weight of each animal was daily measured and recorded before each of the surgical procedures or behavior tests during the 18 experiment day. Seven days after tGCI/R, home-cage chow consumption over a 24 h period was measured in solitary housed rats for one week. During this period, a pre-weighed food ration was provided every morning (8:00 a.m.) and daily food intake was calculated by subtracting the amount of remaining food (including pieces inside the cage) from the pre-weighed food ration 24 h later.

2.5. Neurological deficits evaluation

Neurological deficit score was applied to assess post-stroke behavioral recovery according to a standard scoring system (Akdemir et al., 2014). Briefly, in a quiet comfortable environment, animals were observed and a neurological deficit score ranging from 0 to 12 was assigned, with the maximum score of 12 represented a normal/sham surgery rat and the minimum score of 0 indicated a live but comatose (severe injury) rat at several time points after tGCI/R. The scoring system includes six evaluation indicators: level of consciousness, corneal reflex, respiration, righting reflex, coordination and movement/activity, and each indicator scored 0, 1 or 2. After completing neurologic score evaluation, the score for each animal was calculated by summing the individual scores. The evaluation was performed by an experimenter who was blind to the experimental design.

2.6. Behavioral test and analyses

Behavioral tests were conducted in the order as depicted in Fig. 1 A within 2 weeks after tGCI/R in a soundproof room. To minimize possible stressful effects of prior testing, there was a one-day break between two tests, and all of the behavioral tests were performed during the light phase of the light/dark cycle (9:00 a.m.-2:00 p.m.) with an order from the least to most stressful. All behavioral tests were

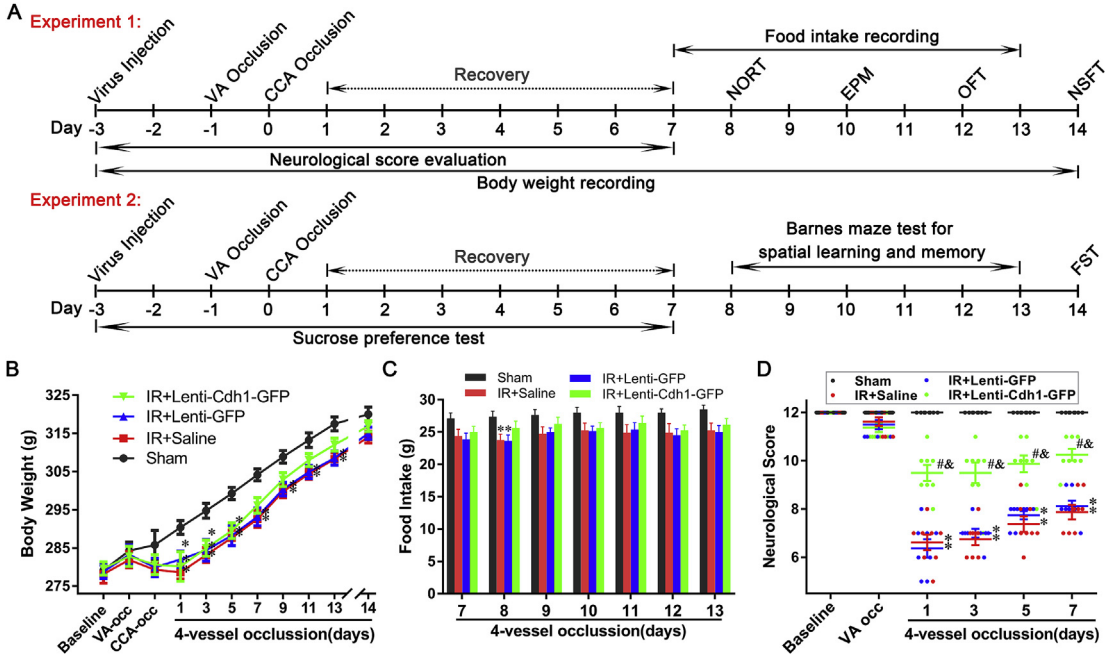

Fig. 1. Experimental design and effect of Cdh1 overexpression on body weight gaining, food intake and neurological outcome. (A) Timeline for the battery of behavioral assays following lentiviral injection and tGCI/R. Hippocampal lentiviral vectors were microinjected three days before 4-VO, neurological score and sucrose preference index were evaluated during one-week recovery period. In experiment 1, novel object recognition test (NORT), elevated plus maze (EPM), open field test (OFT) and novelty suppressed feeding test (NSFT) were used to evaluate recognition, locomotion, anxiety and depression related behaviors on days 8, 10, 12 and 14 post ischemia respectively. In experiment 2, Barnes maze was employed to evaluate cognition 8–12 days after 4-VO and FST was used to assess depressive-like behavior on day 14 post 4-VO. VA, vertebral artery; CCA, common carotid artery. (B). Body weight at baseline, VA occlusion and 2 weeks post 4-VO, Cdh1 overexpression partially reversed tGCI/R induced decrease of body weight gaining. (C) The effect of Cdh1 overexpression on food consumption after tGCI/R in rats. (D) Cdh1 overexpression improved neurological outcome of tGCI/R rats. Values represent mean ± SEM, n = 8 per group, * $P < 0.05$ versus Sham; # $P < 0.05$ versus IR + Saline, & $P < 0.05$ versus IR + Lenti-GFP.

performed by an experimenter who was blind to the experimental design.

2.6.1. Elevated plus maze

Anxiety-like and exploratory behaviors were investigated using elevated plus maze (EPM) as previously described (Lu et al., 2016). The EPM apparatus consisted of two opposing open arms (50 cm × 10 cm with a 5 mm Plexiglas lip) and two opposing closed arms (50 cm × 10 cm with 40 cm high walls) with an open 10 cm × 10 cm area in the center. The entire maze was elevated 60 cm from the floor, and surrounded by a curtain. Each rat was placed into the central square facing an open arm and allowed to freely explore the maze for 5 min, the movement and exploring performance were recorded using an overhead automatic video tracking camera fixed to the ceiling. The head dipping behaviors and entries into or time spent in the open or closed quadrants were analyzed using automatic animal behavior analysis software (Yihong Technology, Wuhan, China). After each test, the apparatus was wiped using 70% ethanol and allowed to dry before next animal.

2.6.2. Open field test

Open field test (OFT) was employed to assess locomotion, exploration activity and anxiety-like behaviors in line with published protocols (Morgan et al., 2018). The apparatus consisted of a wooden box with 40 cm walls and the bottom (100 cm × 100 cm) painted black with non-toxic dye. YH-LA software (Yihong Technology, Wuhan, China) was used to divide the arena into 25 grids (5 × 5), and the middle nine grids (3 × 3) were considered as central zone. Rats were gently placed into the center of the field, and spontaneous locomotor activities including total distance traveled, velocity, number of rearing and defecations, and time spent in the center or corner zones of the field were recorded by an overhead automatic video tracking system for 5 min. The field was cleaned with 70% ethanol (v/v) to remove potential residual odor. Total distance traveled was used as an index to evaluate locomotion, and percent time spent in central zone were calculated (time in central zone/5 min × 100%).

2.6.3. Sucrose preference test

Sucrose preference test (SPT) was used to assess anhedonia using a two-bottle free-choice method as previously described (Mouri et al., 2012). Briefly, 6 days after arrival, rats were housed solitarily and trained to consume water from two bottles of 1% sucrose solution (w/v) for 24 h. The next day, a bottle of 1% sucrose solution was replaced with tap water and the basal sucrose preference index was evaluated. For the next observation period, rats were provided with a free choice from two bottles containing 1% sucrose or tap water for 10 consecutive days, with bottles' position being switched every 12 h to prevent potential location preference for drinking. Total liquid consumption was determined by weighing the bottles prior and every 24 h post-exposure. Sucrose preference index was used as a measure for the rats' sensitivity to reward and anhedonia was inferred from reduced sucrose preference in rats (sucrose preference index = sucrose solution consumption/(sucrose solution consumption ± tap water intake) × 100%).

2.6.4. Novelty suppressed feeding test

Novelty-suppressed feeding test (NSFT) was performed on day 14 after tGCI/R as described previously (Li et al., 2016). Rats were fasted for 24 h but with free access to water before NSFT, and the test were conducted in a larger plastic box (40 cm × 40 cm × 30 cm) with floor covered with 2 cm of autoclaved wooden shaves and a light beaming (450 lx) to brightly illuminate the center zone. A single weighed food pellet (regular rat chow) was placed on a filter paper positioned in the center of the box. Individual rat was placed in a corner of the box and allowed to search the food pellet for a maximum of 10 min. The time

that rats taken to bite the food pellet (defined as the rat sitting on its haunches and biting the food pellet with the aid of forepaws) was noted by an experimenter using a stop-watch. Immediately afterward the rat began to bite food pellet, it was transferred to its home-cage, and the amount of food consumed in the subsequent 5 min is measured.

2.6.5. Forced swim test

Forced swim test (FST) was employed to quantify depression-like behavior in tGCI/R rats as described previously (Ge et al., 2013). Briefly, rats were placed into a clear Plexiglas cylinder (25 cm in diameter and 60 cm high) filled with 30 cm depth of water (23–25 °C) for 6 min. The latency for rats to immobile, total time and frequency of immobile, swimming and struggling were recorded. Immobility was defined as floating with or without small movements that contribute to maintaining equilibrium, but that do not contribute to forward movement as in swimming or struggling. Swimming was defined as the performance of active swimming or circular movements. Struggling was defined as the performance of active movements with the forepaws in and out of the water along the side of the swim chamber. Rats were removed from the cylinder, dried with a tower, and returned to their home-cage immediately after FST.

2.6.6. Novel object recognition test

Eight days after tGCI/R, novel object recognition test (NORT) was employed to test recognition ability of rats. The apparatus consisted of an opaque acrylic box with bottom 50 cm × 50 cm and 40 cm high walls. The procedure include 3 phases: environment habituation, object familiarization, and novel object recognition as described previously (Ashabi et al., 2017) with some modifications. Being adapted to the environment for 30 min, individual rat was placed into the empty box for environment habituation (3 min), then for object familiarization (10 min) with two fixed identical objects. Object exploration was defined when the rat's nose was in close proximity (< 2 cm) to the object while the vibrissae were moving, but not when the body of the rat touched the object while the head was pointed to another direction. One hour after familiarization trial, rats were reintroduced to the arena for NORT with one object alternatively replaced with a novel one. Object exploration time was recorded for 5 min and novel object preference index was calculated as novel object exploration time/(novel object exploration time ± familiar object exploration time) × 100%. After each trial, the arena floor and objects were wiped with 70% ethanol to eliminate potential odor cues for the next subject.

2.6.7. Barnes maze

Barnes maze was used to evaluate spatial learning and memory, and cognitive flexibility in rats as previously described (Lu et al., 2016; Rosenfeld and Ferguson, 2014). The apparatus is a well-lit circular black platform, 100 cm in diameter with 20 evenly spaced 10 cm diameter holes around the circumference with an escape tunnel (20 × 15 × 12 cm) located underneath one of the holes and placed on a tabletop 100 cm above the floor, and the schematic diagram was shown in Fig. 6A.

Training phase: On day 8–12 post-ischemia, rats were trained to locate the escape tunnel, which was used as a measure of spatial learning. Briefly, on each training day, rats were placed in the center of the platform and covered with a black removable chamber for 15 s. Then rats were given 4 min to search and learn the location of the escape tunnel, during this period, bright light (aversive noxious stimuli, 1000 lux) was used to increase the incentive to find the escape tunnel. Rats that failed to locate the escape tunnel were gently guided to its location and allowed to residing in the tunnel for 30 s before returned to their home-cage. The latency to locate the escape tunnel and the number of nose pokes into holes that did not contain the escape tunnel was recorded by a video camera fixed to the room ceiling.

Probe trial: Two hours after the final training on day 12, the escape tunnel was removed and rats were allowed to search the maze for 3 min to evaluate long-term memory, latency to reach the previous location of the escape tunnel, time spent and holes searched in the target quadrant were measured, search strategies (random or system) were also analyzed. The maze was thoroughly cleaned with 75% ethanol and dried with towels between trials to remove olfactory cues.

2.7. Golgi-Cox staining

After behavioral tests, the dendrites and dendritic spines were evaluated using rapid Golgi-Cox staining kit (Hitobiotec, Kingsport, TN, USA) strictly according to the manufacturer's structions. Briefly, rats were decapitated and whole brains were immersed in impregnation solution (a mixture of solution 1 and 2) and held in dark at room temperature (23 ± 2 °C) for 14 days (the solution was changed once after 24 h). Then brains were transferred into solution-3 and stored in dark for 3 days at 4 °C (the solution was changed once after 12 h). Brains were dried and quick-frizzed in pre-cooled isopentane (−80 °C) after wrapping with OCT, sectioned at 60 μm with a cryostat (Leica CM 1900, Wetzlar, Germany) and mounted on gelatinized slides. The slides were stained and further processed according to the manufacturer's instructions and then cover-slipped with neutral balsam aqueous mounting medium (36313ES60; Yeasen Biotech, China) and observed with light microscope (Leica DM2500, Wetzlar, Germany) with the help of oil immersion objective. For dendrites and spines analysis, a specified 100-μm-long apical dendritic section of 10 randomly selected pyramidal cells was examined in hippocampal CA1 area and the images were studied using Image J software (NIH, Bethesda, Maryland, USA). The numbers of spines on the 100-μm-long captured sections were counted visually and separately by three experimenters blinded to the experimental design.

2.8. Transmission electron microscopy (TEM)

Hippocampal synapses were examined by TEM after behavior test as described in our previous study (Zhang et al., 2018). Briefly, deep anesthetized rats (n = 4 per group) were transcardially perfused with pre-cooled heparinized saline, followed by a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4). After perfuse-fixation, hippocampal blocks were further fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer overnight at 4 °C. Then samples were impregnated in 1% osmium tetroxide for 2 h, dehydrated in a series of graded acetone and then embedded in Epon 812 medium. For solidification, the tissues were placed into a dry oven overnight at 37 °C, followed by 60 °C for 12 h. Thereafter, ultra-thin sections of each sample were acquired using a diamond knife, sections were double-stained with uranyl acetate and synapses were analyzed using a transmission electron microscope (HT770 0, Hitachi, Tokyo, Japan). Approximately 15–20 pictures were taken for each group of 4 different samples. All synapses were analyzed and counted by two experimenters blind to sample information according to the method used in a previous publication (Ortiz-Matamoros and Arias, 2018).

2.9. Western blot analysis

Western blot was performed according to protocols used in our lab after behavioral tests. Briefly, hippocampal samples were fully homogenized with Teflon-glass homogenizer in pre-cooled lysis buffer consisting of 1 mM PMSF and RIPA. After sonicating in 10-s pulses 3 times, the lysates were centrifuged at 12,000 rpm for 30 min at 4 °C and supernatants were collected. Protein concentration was determined using BCA Kits (Pierce, Rockford, IL, USA) and samples were boiled at 95 °C in SDS loading buffer for 8 min and stored at −80 °C until use. Equal amount of protein (40 μg/lane) were electrophoresed by 10–13% SDS-PAGE gels and electro-transferred onto 0.45 μm PVDF membranes. Membranes were then blocked in Tris-buffered saline (TBS) including 5% skim milk for 90 min at room temperature (23 ± 2 °C) with gentle

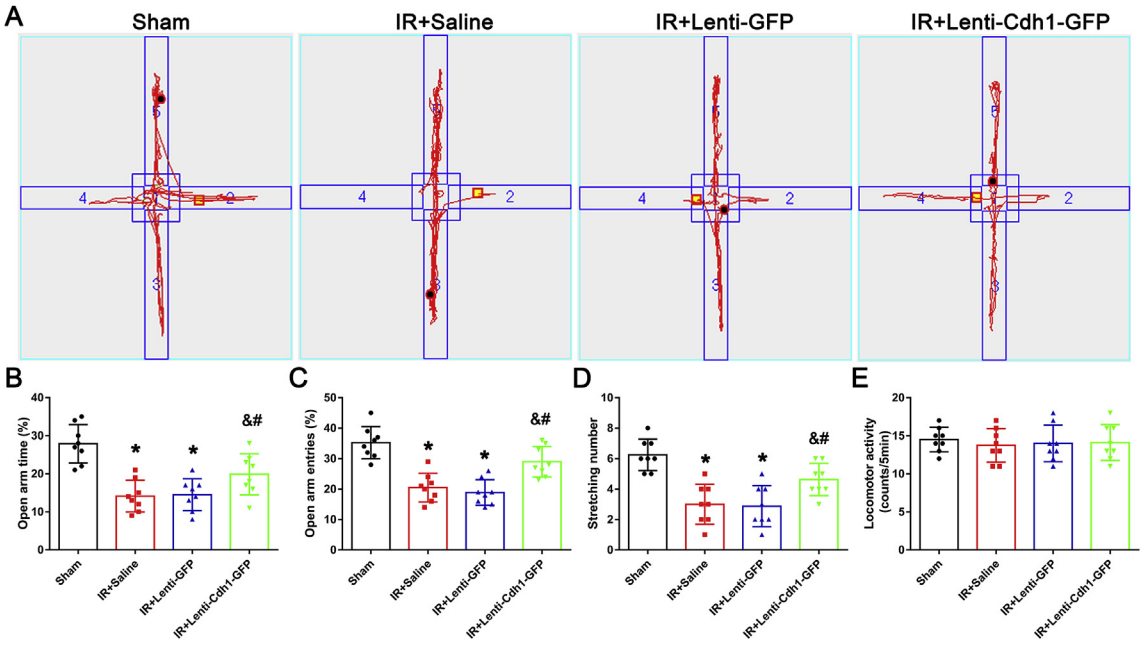

Fig. 2. Cdh1 overexpression relieves the anxiety of tGCI/R rats in the elevated plus maze. (A) Representational trajectories of rats moving in the EPM. (B) Percentage of time rats moving/staying in the open arms. (C) Times that rats entered the open arms during the 5 min EPM test. (D) Times that rats stretching during the 5 min EPM test. (E) Total number of grids rats crossed during the 5 min EPM test. Data are presented as mean ± SD, n = 8 per group. * $P < 0.05$ versus Sham, # $P < 0.05$ versus IR + Saline, & $P < 0.05$ versus IR + Lenti-GFP.

shaking, and then incubated overnight at 4 °C with primary antibodies diluted in blocking buffer: rabbit anti-GAP-43 (1:1000; ab75810, Abcam, Cambridge, UK), mouse anti-synaptophysin (1:1000; Millipore, Temecula, CA, USA), rabbit anti-synapsin (1:1000; Abcam, Cambridge, UK), mouse anti-PSD95 (1:2000; Cell Signaling Technology, Danvers, MA, USA), or mouse anti-β-actin (1:500, Boster, Wuhan, China). The next day, membranes were washed 3 × 10 min with 0.1% Tris-buffered saline/Tween 20 (TBST), incubated with HRP-conjugated secondary antibody (1:5000, Boster, Wuhan, China) for 1 h at room temperature (23 ± 2 °C), and rinsed 4 × 10 min with 0.1% TBST subsequently. Finally, protein band intensities of membranes were detected using ECL kit (Thermo Scientific, USA) and Chemi-Doc XRS imaging system (Bio-Rad, CA, USA). The quantitation analysis was performed and relative protein expression levels were compared to the loading control protein β-actin.

2.10. Statistical analysis

The normal distribution of behavioral data was assessed using the D'Agostino-Pearson test. All results were analyzed using GraphPad Prism software version 7.0 (Graphpad Software, Inc., San Diego, CA, USA) and values are expressed as the mean ± S.E.M. or mean ± SD. One-way analysis of variance or repeated measures of two-way analysis of variance and post-hoc Tukey's multiple comparison tests were used for normally distributed data, and the Mann-Whitney U tests were used for neurological score analysis. $P < 0.05$ were considered statistically significant.

3. Results

3.1. Recovery of decreased body weight and food intake after ischemia

The impact of tGCI/R and virus injection on body weight gaining was determined during the experiment (Fig. 1B). There were no significant differences among body weight of rats in all groups at initial baseline and before bilateral CCAs occlusion, however, tGCI/R rats showed reduced body weight gaining as compared to their counterparts in Sham group from the first day on after ischemia. Repeated measures of two-way ANOVA revealed an effect of time [$F_{(10, 280)} = 496.5$, $P < 0.0001$] and treatment [$F_{(3, 28)} = 4.174$, $P = 0.0146$], and there was also an interaction effect of time and treatment [$F_{(30, 280)} = 2.625$, $P < 0.0001$]. Simultaneously, the impact of tGCI/R on individual rat food intake was monitored one week after reperfusion for a consecutive seven days. As shown in Fig. 1C, tGCI/R rats showed reduced daily food consumption when compared to their counterparts in Sham group from post-ischemic day 7–13, with an exception for the 9th day (no statistical significant difference in food consumption of rats in each group, $P > 0.05$).

3.2. Cdh1 overexpression improves neurological deficit score after tGCI/R

Neurological outcome of surviving rats, as assessed using a standard neurological scoring system, revealed that all animals displayed the maximum score of 12 points without neurological impairment at baseline, whereas it decreased significantly in IR + Saline and IR + Lenti-GFP group rats when compared to rats in Sham group after 4-VO (all $P < 0.05$), and this decrease was significantly improved in Lenti-Cdh1-GFP-treated rats, with fairly stable neurological scores from day 1–5 after 4-VO (Fig. 1D). Vertebral-artery occlusion produced a consistent mild ataxia in all rats exposed to tGCI/R (neurological score reduced from 12 to 11) as expected. Little further reduction in neurological score was seen in Saline and Lenti-GFP-treated rats after transient bilateral CCA occlusion. In addition, the decrease of neurological score of tGCI/R rats could gradually recover within one week after ischemia.

3.3. Effects of Cdh1 overexpression on locomotion and anxiety-like behaviors

3.3.1. Elevated plus maze

EPM was used to analyze anxiety-related behavior based on a preference for rodents to explore and spend time in a 'safer' environment of the closed versus the open arms of the maze (Cryan and Holmes, 2005). Representative trajectories of rats moving in the EPM were shown in

Fig. 2A, according to statistical results, percent time that rats spent in the open arms (Fig. 2B) in IR + Saline group (14 ± 4.2%) and IR + Lenti-GFP group (15 ± 4.2%) were significantly less than that of Sham group (28 ± 5.1%), whereas Cdh1 overexpression prolonged the percentage of time (20 ± 5.4%) that tGCI/R rats spent in open arms, one way-ANOVA showed a treatment effect [$F_{(3,\ 28)} = 14.76$, $P < 0.0001$]. Similar to the tendency of time spent in open arms, percent frequency of open arm entries in IR + Saline group (21 ± 4.7%) and IR + Lenti-GFP group (19 ± 4.2%) were significantly less than that of Sham group (35 ± 5.3%), whereas this trend was partially reversed in IR + Lenti-Cdh1-GFP group rats (29 ± 5%) (Fig. 2C). Total stretching number (an exploring indicator) of rats in IR + Saline group (3 ± 1.3) and IR + Lenti-GFP group (2.9 ± 1.4) were significantly less than that of Sham rats (6.3 ± 1), and this was also reversed in IR + Lenti-GFP group rats (4.6 ± 1.1) (Fig. 2D). During the 5 min EPM test, total number of entries into closed and open arms showed no significant differences among all groups (Fig. 2 E).

3.3.2. Open field test

Animal activities in open field are often used to evaluate locomotion and anxiogenic behaviors (Morgan et al., 2018). The representational trajectories of rats moving in the open field were shown in Fig. 3A. Mean total time of rats spent at the center (Fig. 3B) in IR + Saline (75 ± 13 s) and IR + Lenti-GFP (72 ± 12 s) group were significantly shorter than that of their counterparts in Sham group (132 ± 19 s), whereas Cdh1 overexpression partially reversed the shortening of rat activity time in the central area (126 ± 16 s), one way-ANOVA showed a treatment effect [$F_{(3,\ 28)} = 36.6$, $P < 0.0001$]. Simultaneously, the change trend of time rats spent at the corner was opposite to that in the center area (Fig. 3C). The rearing number (Fig. 3D) and grooming number (Fig. 3E) in IR + Saline and IR + Lenti-GFP group rats were both less than that of rats in Sham group, whereas the decreasing tendency of rearing or grooming number was significantly reduced in IR + Lenti-Cdh1-GFP group rats (all $P < 0.01$). As for locomotor activity, tGCI/R rats treated with saline, Lenti-GFP or Lenti-Cdh1-GFP showed no significant differences in the total distance traveled (Fig. 3 F) and velocity (Fig. 3 G) when compared with Sham group rats (all $P > 0.05$).

3.4. Cdh1 overexpression alleviates depression-like behaviors induced by tGCI/R

3.4.1. Sucrose preference test

As shown in Fig. 4A, there were no significant differences as for sucrose preference index among rats in the four groups at baseline (rats that without CCAs occlusion). Whereas the sucrose preference index of tGCI/R rats was remarkably lower than that of their counterparts in

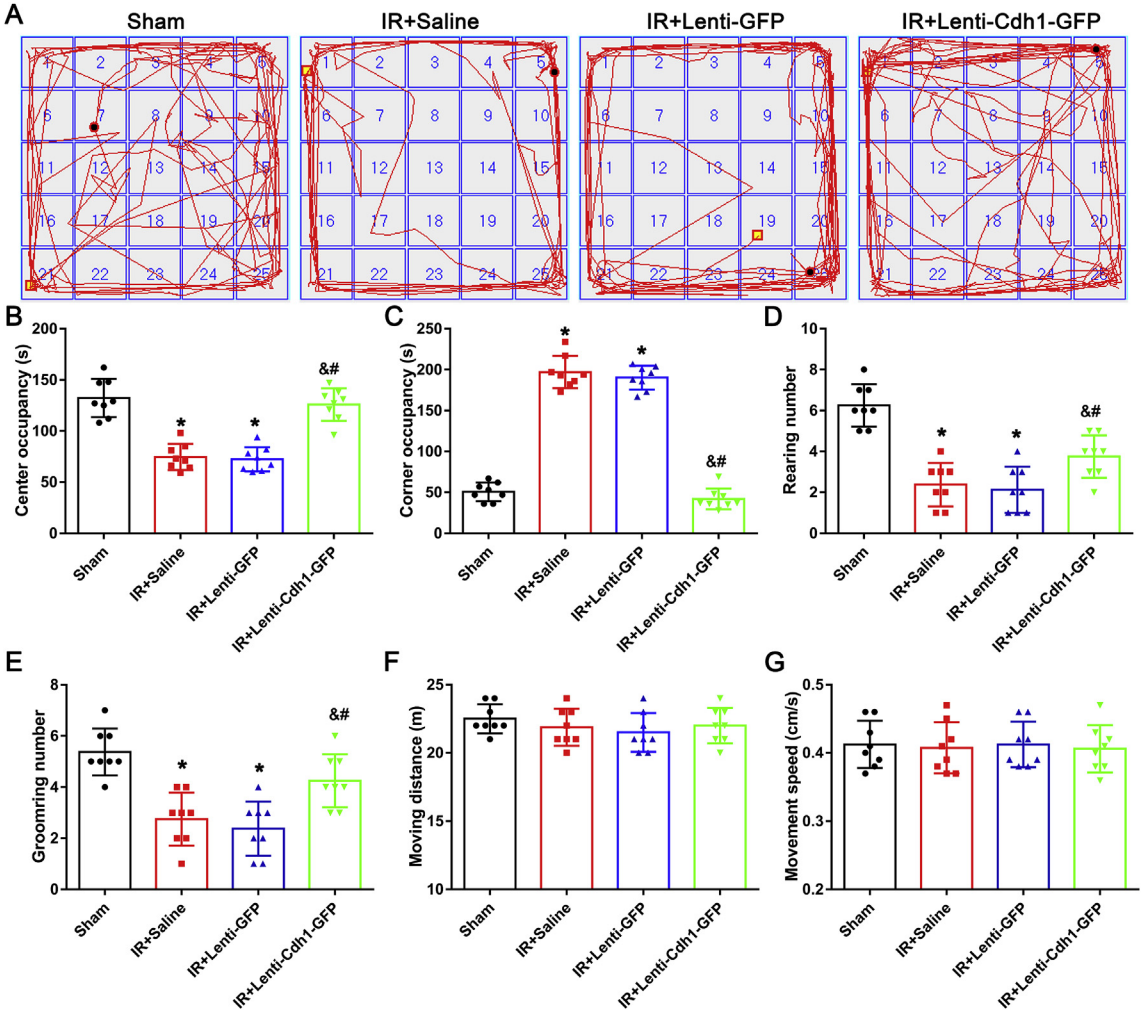

Fig. 3. Cdh1 overexpression relieves the anxiety of tGCI/R rats in the open field. (A) Representational trajectories of rats moving in the open field. (B) Total time of rats spent in the center area of the open field during the 5 min OFT. (C) Total time of rats spent in the corner area of the open field during the 5 min OFT. (D) Times that rats rearing during the OFT. (E) Times that rats grooming during the OFT. (F) The total distance of rats moved during the OFT. (F) Average moving speed of rats during the OFT. Data are presented as mean ± SD, n = 8 per group. * $P < 0.05$ versus Sham, # $P < 0.05$ versus IR + Saline, & $P < 0.05$ versus IR + Lenti-GFP.

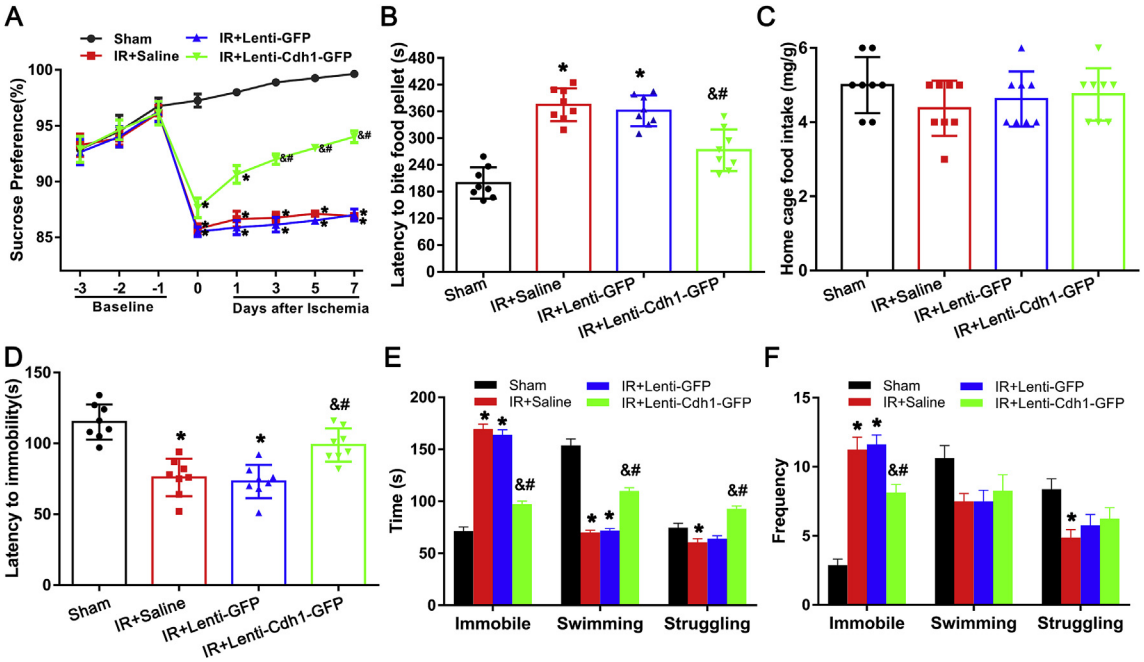

Fig. 4. Cdh1 overexpression improves depression-like behaviors of tGCI/R rats. (A) Changes of sucrose preference index of rats before or within a week after 4-VO. (B) Time that rats spent to find and bite food in a novel environment. (C) Total food consumed in the home cage after food pellet finding. (D) Latency of rats to stop moving/struggling in the FST. (E) Total time of rats spent resting/swimming/struggling in the FST. (F) Frequency of resting/swimming/struggling of rats in the FST. Data are presented as mean ± SEM (A, E and F) or mean ± SD (B, C and D), n = 8 per group. *$P < 0.05$ versus Sham, #$P < 0.05$ versus IR + Saline, &$P < 0.05$ versus IR + Lenti-GFP.

Sham group after 4-VO, and this decreasing trend continued until post-ischemia day 7. However, the decline of sucrose preference index of rats in IR + Lenti-Cdh1-GFP group were significantly alleviated on post-operative day 5, 6 and 7 when compared to their counterparts in IR + Saline or IR + Lenti-GFP group at the same time point (all $P < 0.05$). It is noteworthy that there were no significant differences in total daily fluid consumption among the rats in all groups (data not shown).

3.4.2. Novelty suppressed feeding test

The effect of Cdh1 overexpression on feeding in novel environment was test using the NSFT. One-way ANOVA revealed a significant effect of treatment [$F_{(3,28)} = 36.21$, $P < 0.0001$] on feeding latency (Fig. 4B). Tukey's multiple comparisons test indicated that, compared with Sham rats, the feeding latency of rats in Saline or Lenti-GFP-treated tGCI/R rats were significantly increased (the feeding latency of the three groups were 200 ± 35 s, 375 ± 37 s and 362 ± 35 s), whereas Lenti-Cdh1-GFP-treated rats showed reduced feeding latency (273 ± 47 s) in NSFT ($P < 0.0001$ vs IR + Saline, $P = 0.0005$ vs IR + Lenti-GFP). However, there were no significant differences as for home cage food consumption among the four groups [$F_{(3,28)} = 0.9945$, $P = 0.4098$] as depicted in Fig. 4C.

3.4.3. Forced swim test

FST is an inescapable stressful experience, so the latency of the first time animals give up struggling/swimming and the overall amount of time they spent keeping immobile, struggling or swimming are the main measures of depression-like behavior (Rogers et al., 2017). One-way ANOVA revealed a significant effect of treatment [$F_{(3,28)} = 21.15$, $P < 0.0001$] on immobility latency (Fig. 4D), the latency to immobility of rats in IR + Saline group (76 ± 13 s) and IR + Lenti-GFP group (73 ± 12 s) were significantly shorter than that of rats in Sham group (115 ± 12), whereas this latency extended to 99 ± 12 s in IR ± Lenti-Cdh1-GFP treated rats. The overall amount of time rats spent keeping immobile in Sham rats was 71 ± 11 s, and this was significantly prolonged in IR + Saline (170 ± 13 s) and IR + Lenti-GFP group (164 ± 13 s), whereas Cdh1 overexpression reversed this trend to 97 ± 8.6 s significantly (all $P < 0.05$) (Fig. 4E). The trend of overall time rats spent swimming or struggling were consistent with that of immobility, with 154 ± 18 s, 70 ± 6.4 s, 72 ± 5.9 s and 110 ± 9.3 s for swimming, and 75 ± 12 s, 61 ± 10 s, 64 ± 8.1 s and 93 ± 7.6 s for struggling, respectively. The frequencies of rats keeping immobile, swimming or struggling were also analyzed, as depicted in Fig. 4F, the immobile frequency of rats in IR + Saline group (11 ± 2.5) and IR + Lenti-GFP group (11 ± 1.9) were significantly higher than that of rats in Sham group (2.9 ± 1.2), whereas Cdh1 overexpression reversed this to 8.1 ± 1.7 and the difference was statistically significant when compared to IR + Saline or IR + Lenti-GFP group (all $P < 0.05$). As for the frequency of swimming and struggling, differences among groups were not statistically significant, but Cdh1 overexpression has a tendency to increase the frequency of swimming or struggling of tGCI/R rats in FST.

3.5. Effects of Cdh1 overexpression on cognitive-related behaviors

3.5.1. Novel object recognition test

NORT was performed with three phases as depicted in Fig. 5A. The exploration time of rats on different objects during object familiarization and novel object recognition period was recorded and the novel object index was calculated. As shown in Fig. 5B, there were no significant differences in exploration time between object A and object B (same size and shape) among all groups ($P > 0.05$) in object familiarization period. However, during recognition phase (Fig. 5C), the percentage of exploration time differences between object A or B (familiar object) and the novel object C in Sham and IR ± Lenti-Cdh1-GFP group with longer exploration time for novel object (all $P < 0.05$), whereas there were no significant statistical differences between the familiar and the novel object in IR + Saline and IR + Lenti-GFP group ($P > 0.05$). One-way ANOVA revealed a significant effect of treatment [$F_{(3,28)} = 15.3$, $P < 0.0001$] on novel object recognition index. As shown in Fig. 5D, the novel object preference index in IR + Saline (45 ± 9.4%) and IR + Lenti-GFP (43 ± 6.6%) were significantly

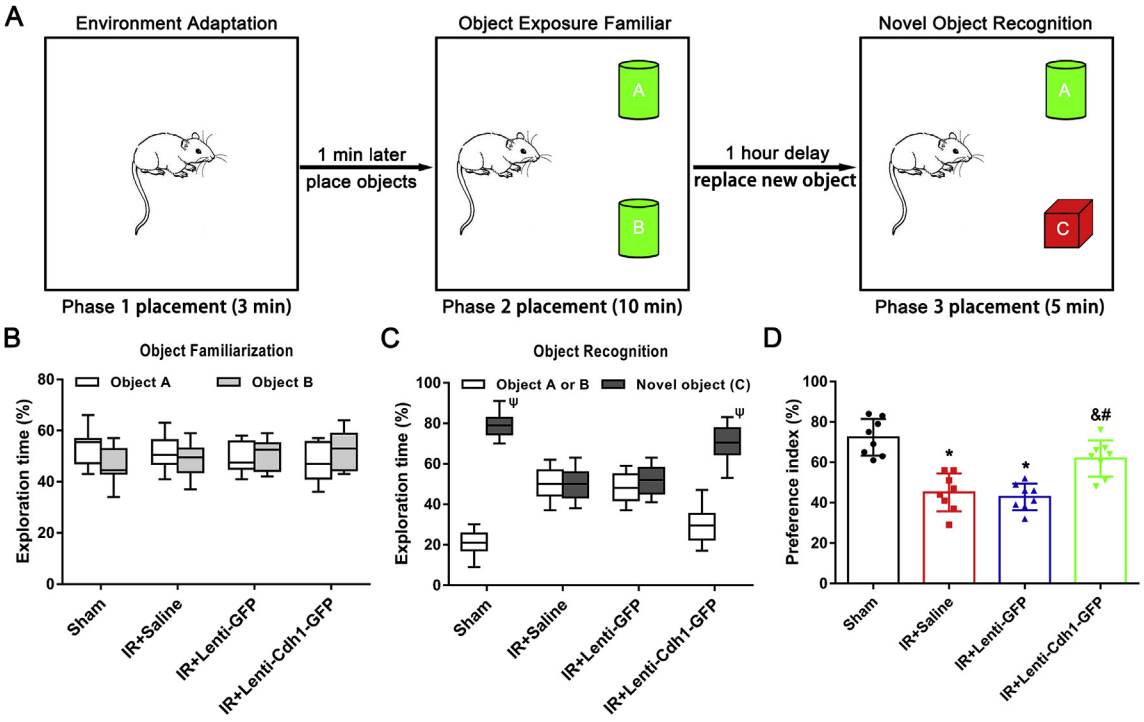

Fig. 5. Cdh1 overexpression improves recognition ability of tGCI/R rats. (A) Schematic diagram of NORT; (B) Time of rats spent exploring two identical object A and B during the period of familiarization. (C) Time of rats spent exploring old object A or B and novel object C during the period of recognition. (D) Novel object preference index of rats during the period of recognition. Data are presented as mean ± SD, n = 8 per group. ψ $P < 0.05$ versus old object A or B, * $P < 0.05$ versus Sham, # $P < 0.05$ versus IR + Saline, & $P < 0.05$ versus IR + Lenti-GFP.

lower than that of Sham rats (72 ± 9.1%) (all $P < 0.05$), whereas the novel object preference index in IR + Lenti-GFP group (62 ± 9%) was higher than that of IR + Saline or IR + Lenti-GFP groups (all $P < 0.05$).

3.5.2. Barnes maze test

It has been widely acknowledged that hippocampus plays an important role in the processing of spatial locations (Hunsaker and Kesner, 2018). Repeated measures of two-way ANOVA (group × day) for latency to escape tunnel during BM acquisition revealed significant main effects for group and for day [group $F_{(3, 21)} = 4.028$, $P = 0.0207$; day $F_{(4, 28)} = 730.5$, $P < 0.0001$; interaction $F_{(12, 84)} = 3.629$, $P = 0.0002$] (Fig. 6B), we also observed significant group, day and day × group effects among the four experimental groups for total distance traveled (Fig. 6C) and number of searching errors (Fig. 6D). The latency, distance traveled and searching errors of rats in IR ± Lenti-Cdh1-GFP group to find the escape tunnel were significantly less than that of rats in IR + Saline or IR + Lenti-GFP group (all $P < 0.05$). The representative trajectories of rats to locate the escape tunnel on day 12 were shown in Fig. 6E.

Two hours after the last training, a probe trial was conducted without escape tunnel to quantify spatial retention memory. As shown in Fig. 6F, rats in Sham group and IR ± Lenti-Cdh1-GFP group prefer to use systematic search strategies, whereas there were not much differences in the proportion of using two search strategies of rats in IR + Saline and IR + Lenti-GFP group ($P > 0.05$). Time spent in the target quadrant (Fig. 6G) of rats in IR + Saline group (30 ± 4 s) and IR + Lenti-GFP group (29 ± 3.5 s) were significantly less than that of rats in Sham group (63 ± 5.3 s), however, this tendency was reversed in Lenti-Cdh1-GFP-treated tGCI/R rats (43 ± 5.3 s) ($P < 0.05$). Similar to this, percent holes searched in the target quadrant (Fig. 6H) of rats in IR + Saline group (21 ± 4.5%) and IR ± Lenti -GFP group (21 ± 4.8%) were less than that of rats in Sham group (56 ± 4.9%), and this was reversed in Lenti-Cdh1-GFP-treated tGCI/R rats (38 ± 4.9%) ($P < 0.05$). The representational trajectories intuitively show the differences in spatial memory of the rats during the test period (Fig. 6I).

3.6. Effects of Cdh1 overexpression on hippocampal neuroplasticity

Golgi-Cox staining was used to detect changes in morphology of dendrites and dendritic spines, representational images showed that hippocampal neurons are clearly stained brown and black, and can be followed in the entire length, the spines of the apical dendrites can be detected under light microscope (Fig. 7A). Statistical results showed that in the range of 20–40 μm from the soma, the dendritic branches in Saline or Lenti-GFP treated tGCI/R rats were significantly less than that in Sham rats, whereas Cdh1 overexpression reversed the reduction of dendritic branches induced by tGCI/R (Fig. 7B). It also produced a certain impact on the length of dendrites, the average length of the dendrites in IR + Saline group (53 ± 13 μm) and IR + Lenti-GFP (50 ± 14 μm) group were significantly shorter than that of Sham group (108 ± 17 μm), whereas this shortening was reversed to 80 ± 8.2 μm in Lenti-Cdh1-GFP group (Fig. 7C). Similarly, Cdh1 overexpression increased the number of dendritic spines in tGCI/R rats, the spine density in the four groups were 2.2 ± 0.3, 1.3 ± 0.24, 1.2 ± 0.18, 1.9 ± 0.29/μm individually, and significant differences were observed between Lenti-Cdh1-GFP-treated group and the Saline- or Lenti-GFP-treated group (all $P < 0.05$) (Fig. 7D).

We further employed TEM to investigate changes in the number of hippocampal synapses, similar to the results of Golgi-cox staining, representative TEM images visually showed that Cdh1 could partially reversed the reduction of hippocampal synapses induced by tGCI/R (Fig. 7E) and the statistical results revealed that the number of hippocampal synapses (area of counting frame is 3.2 μm) in IR + Saline group (40 ± 4.55) and IR + Lenti-GFP group (38.5 ± 4.8) were significant less than that in Sham group (63.25 ± 6.02), whereas this in Lenti-Cdh1-GFP-treated group (53.5 ± 4.66) was significantly

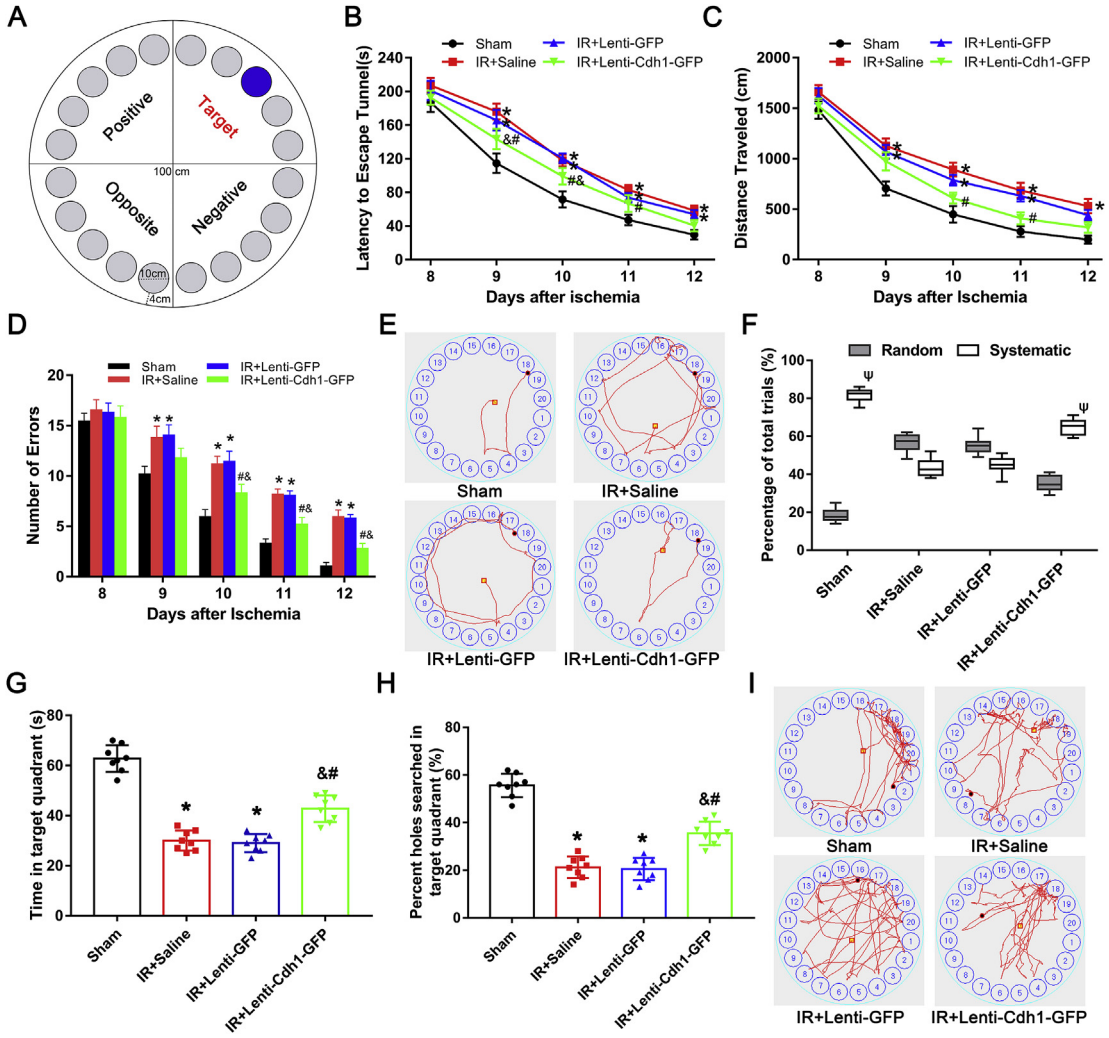

Fig. 6. Cdh1 overexpression attenuates learning and memory deficits of tGCI/R rats. (A) The schematic diagram of the zone and dimensions of Barnes maze apparatus. (B) The average latency of rats to find the black escape tunnel at day 8–12 after tGCI/R. (C) The average distance of rats traveled to find the escape tunnel at day 8–12 after tGCI/R. (D) Total number of errors during the 5 training days. (E) Representative trace image of rats to find the escape tunnel on day 12 after tGCI/R. (F) Search strategy of rats during the probe trial. (G) Total time of rats spent exploring the target quadrant during the probe trial. (H) Percentage holes searched in the target quadrant during the probe trial. (I) Representative tracks in the quadrant zone during spatial memory probe trial. Data are presented as mean ± SEM (B, C and D) or mean ± SD (G and H), n = 8 per group. ψ $P < 0.05$ versus random, $^{*}P < 0.05$ versus Sham, $^{\#}P < 0.05$ versus IR + Saline, $^{\&}P < 0.05$ versus IR + Lenti-GFP.

increased when compared to IR + Saline or IR + Lenti-GFP group (all $P < 0.05$) (Fig. 7F).

To verify the effects of hippocampal Cdh1 overexpression on synaptic-related proteins, we investigated the relative expression level of GAP-43, synaptophysin (SYP), synapsin (SYN), and PSD-95 in the hippocampus (Fig. 7G–K). When the level of GAP-43 was set at 1.0 in Sham group, the level of GAP-43 was decreased to 0.45 ± 0.13 in Saline-treated group and 0.5 ± 0.13 in Lenti-GFP-treated group; however, this decrease was attenuated (relative expression 0.78 ± 0.10) in Lenti-Cdh1-GFP-treated group. Furthermore, when the level of SYP was set at 1.0 in Sham group, the level of SYP was decreased to 0.5 ± 0.08 in Saline-treated group and 0.4 ± 0.08 in Lenti-GFP-treated group, but the decrease was attenuated (relative expression 0.73 ± 0.1) in Lenti-Cdh1-GFP-treated group. The level of SYN showed the same change trend, that the relative expression level in IR + Saline, IR + Lenti-GFP and Lenti-Cdh1-GFP group was 0.28 ± 0.1, 0.3 ± 0.08, 0.6 ± 0.08 individually. Similar results were observed for the post-synaptic protein, PSD-95 (0.48 ± 0.13 in Saline-treated group, 0.38 ± 0.10 in Lenti-GFP-treated group and 0.8 ± 0.08 in Lenti-Cdh1-GFP-treated group). These histological and molecular biological evidences point to the effect of Cdh1 overexpression on restoration and preservation of neuroplasticity.

4. Discussion

Brain injury after tGCI/R is associated with high morbidity and mortality, and has long-term neurosensory or neurologic sequelae in survivors. Anxiety, depression, and cognitive impairment experienced by post-stroke survivors are some of the most prevalent neuropsychiatric conditions currently requiring therapeutic solutions. The present study investigated the effect of hippocampal Cdh1 overexpression on anxiety-like, depression-like, and cognitive-related behaviors in tGCI/R rats. Our results revealed that hippocampal Cdh1 overexpression could alleviate negative emotionality and improve cognitive dysfunction induced by tGCI/R, and this neuroprotective effect could partially be attributed to the regulation effects of Cdh1 on hippocampal neuroplasticity after ischemia.

Disruption of global cerebral circulation may result in several injuries that would occur in important brain regions such as hippocampus, striatum, prefrontal cortex, and thalamus which are involved

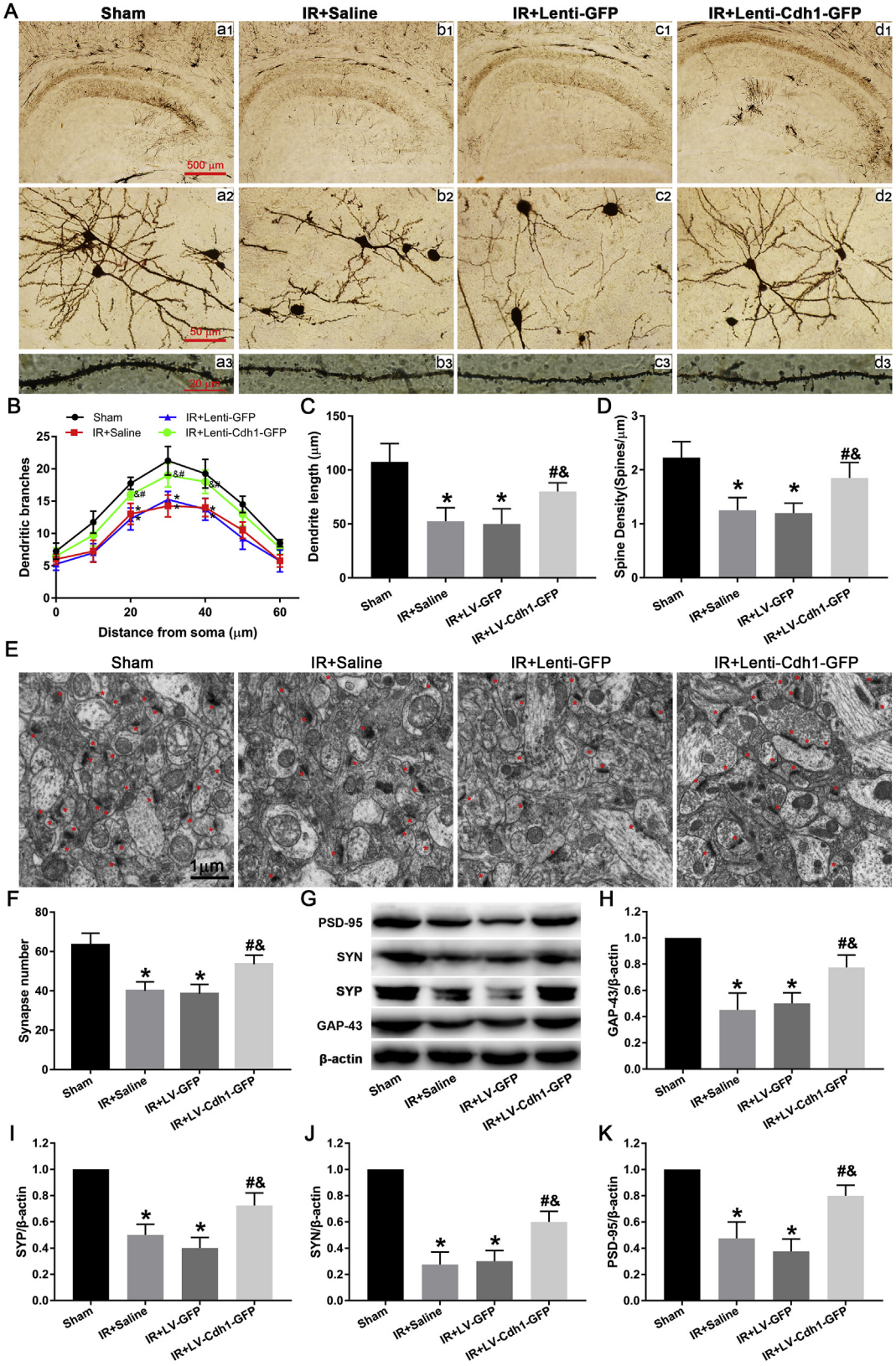

Fig. 7. Cdh1 overexpression affects hippocampal neuroplasticity after tGCI/R. (A) Representative images of dendrites and dendritic spines in each group, Scale bar = 500 μm in the upper row, 50 μm in the middle row and 20 μm in the lower row. (B) Statistical analysis of dendritic branches within 60 μm from the soma. (C) Average dendrite length in the hippocampus of rats in the four groups. (D) Average density of dendritic spines in the hippocampus of the four groups. (E) Representative images of hippocampal synapses under transmission electron microscope. Scale bar = 1 μm. (F) Quantitative statistics of hippocampal synapses in the four group. (G) Representative Western Blot images of protein associated to neuroplasticity. (H–K) The quantitative analysis of protein level of GAP-43, SYP, SYN and PSD-95. Data are presented as mean ± SEM, n = 4 per group. $^{*}P < 0.05$ versus Sham, $^{\#}P < 0.05$ versus IR + Saline, $^{\&}P < 0.05$ versus IR + Lenti-GFP.

in the regulation of emotion, cognition and other neurological functions (Traystman, 2003; Yang et al., 2008). In the current study, tGCI/R rats exhibited significant motor/sensory impairment, negative emotionality, cognitive deficit and showed reduction of body weight gaining without differences in daily food intake. Hippocampus is a part of limbic system which is involved in emotionality, mood, mental sensation and cognition (Ressler and Mayberg, 2007). Cerebral ischemia may cause damages in hippocampal structure and function that would leading to motor/sensory injury and other functional impairments (Akdemir et al., 2014; Ifergane et al., 2018; Lee et al., 2015; Williams et al., 2004). However, hippocampal Cdh1 overexpression partially alleviated motor/sensory injury caused by ischemia, rescued ischemia-induced body weight loss and promoted neurological rehabilitation after ischemia.

Ischemia has more effectual roles on anxiety-related behaviors rather than locomotion, hippocampus is required for anxiety-like behaviors (Adhikari et al., 2010) and it is the most vulnerable brain region during tGCI/R (Pulsinelli and Brierley, 1979). Nevertheless, we have confirmed that hippocampal neuronal death was accompanied by the down-regulation of Cdh1 after tGCI/R (Zhang et al., 2011, 2018). Previous studies indicated that anxiety-related behaviors were increased in ischemia rats (de la Tremblaye et al., 2017; Sarkaki et al., 2015; Zhang et al., 2016), whereas there were still studies confirmed that anxiety-like behaviors were significant decreased (Bantsiele et al., 2004; Plamondon and Khan, 2005) or not altered (Oz et al., 2017) after cerebral ischemia. It is noteworthy that ischemic rats venturing more in the anxiogenic open arms when using an OFT-EPM testing sequence (Knowles et al., 2016). We deduce that these inconsistencies may stem from different animal strains, different ischemic models or different measurement time points. Thus OFT was carried out one day after EPM in our study, and tGCI/R rats exhibited more anxiety-related behaviors both in EPM and OFT, whereas Cdh1 overexpression alleviated tGCI/R induced anxiety-like behaviors. On the other hand, our data demonstrated that tGCI/R has no effect on locomotion which was coincide with others' research (Oz et al., 2017) and our previous MWM results (Zhang et al., 2018).

With respect to depressive-like behavior, decreased anhedonia in SPT and increased despair in FST have been reported 14 days after BCCAO in mice (Lee et al., 2015; Soares et al., 2016). Major depression disorder is a serious illness characterized by fatigue, anhedonia and despair (Mouri et al., 2012). Immobility in FST is considered as a measure of anhedonia or despair, which is the core symptom of depressive disorders. Previous studies indicated that tGCI/R rats showed increased immobility in the FST (Rygula et al., 2005; Slattery and Cryan, 2012), reduced sucrose preference index and prolonged latency to feed in NSFT (de la Tremblaye et al., 2016), we found that tGCI/R rats displayed typical anhedonia and despairing behaviors whereas Cdh1 overexpression effectively attenuated tGCI/R-induced depressive-like behaviors. It is argued that multitude of factors can interfere with animal behaviors in the test of depression and/or anxiety, and the complexity of neurobiology of depression and anxiety may also affect the result of behavior test (Soares et al., 2016; Tiller, 2012). However, our results suggest that Cdh1 may function as a negative factor in the development of depression-like and anxiety-like behaviors, and upregulating Cdh1 level may protect animals from developing negative emotionality after ischemia.

Neuronal damage during ischemia is concomitant with cognitive impairment. As noted in our previous study, 10-min tGCI/R impaired spatial learning and memory in MWM (Zhang et al., 2018). Barnes maze was originally developed by Carol Barnes to overcome stress induced by swimming in the MWM (Barnes, 1979), rodents with hippocampal damage showed impaired performance in Barnes maze (Lu et al., 2016). In the current study, tGCI/R rats took more time and traveled more distance to reach the escape tunnel, which suggesting impaired spatial learning and memory, and this is consistent with previous publications (Ahmed et al., 2017; de la Tremblaye et al., 2017; Zhao et al., 2018). NORT is used for evaluating short-term working memory, concomitant with the results of decreased discrimination index in BCCAO rats (Soares et al., 2016), we found that tGCI/R rats induced a reduction in the exploration time of newly different object. However, Cdh1 overexpression improved ischemia-induced long-term spatial learning and memory impairments in the Barnes Maze and improved short-term working memory in the NORT.

APC/C–Cdh1 is an important E3 ubiquitin ligase (Pick et al., 2013), and it has been linked to diverse neurobiological functions including the regulation of neuronal survival, axonal growth and synaptic plasticity (Fuchsberger et al., 2017; Yao et al., 2010). Ring Finger 41, an E3 ubiquitin ligase, was recognized as a candidate gene for anxiety-like and depression-like behaviors (Kim et al., 2009). Mice postnatal deletion of Cdh1 showed impaired locomotion/explorative activity and higher levels of anxiety (Bobo-Jimenez et al., 2017). Neuron-specific deletion of Cdh1 causes impaired extinction of fear memory (Pick et al., 2013) and Cdh1 cKO mice showed impaired associative fear memory (Pick et al., 2012). All these indicated that Cdh1 may exert a role in emotion and cognition regulation. Actually, Cdh1 participate in the regulation of emotionality and cognition through a variety of different mechanisms (Frey et al., 2015; Huang et al., 2015; Pick et al., 2013). Previous studies identified that tGCI/R induced rapid and sustained reorganization of synaptic structures, damage of dendritic structures and loss of spines in hippocampus (Zhu et al., 2017), Cdh1 deficiency in pyramidal neurons disrupts the dendritic network (Bobo-Jimenez et al., 2017), and we previously confirmed that down-regulation of Cdh1 participate in the process of apoptotic neuronal death and synapse ultrastructure changes after tGCI/R (Zhang et al., 2011, 2018). In present study, we further confirmed that Cdh1 overexpression partially rescued ischemia-induced loss of hippocampal synapses and alleviate the disorganization of dendritic network.

Pre-synaptic proteins like growth associated protein 43 (GAP-43), synaptophysin (SYP), synapsin (SYN) and post-synaptic protein PSD-95 are indicators of synaptic structure and have previously been used to evaluate neuroplasticity in other models of cerebral ischemia (Kinjo et al., 2018). Hippocampal neuroplasticity has been confirmed to associate with behaviors such as anxiety, depression and cognition (Bannerman et al., 2014; Ifergane et al., 2018) and these behaviors are dependent on proper hippocampal circuitry and function (Li et al., 2016). We found that tGCI/R induced markedly decline in the levels of GAP-43, SYP, SYN and PSD-95 in the rat hippocampus, and this was consistent with published results using focal or global cerebral ischemia model (de la Tremblaye et al., 2017; Juan et al., 2014; Li et al., 2017a; Stokowska et al., 2017; Xin et al., 2018), whereas Cdh1 overexpression partially reversed the decrease of neuroplasticity associated proteins. All of these results suggesting that Cdh1 overexpression may exert protective effects through enhancing hippocampal neuroplasticity after tGCI/R.

There are also some limitations in our study. First, we did not carry out electrophysiological experiments to confirm the regulation effects of Cdh1 overexpression on neuroplasticity. Second, we did not confirm mechanisms by which Cdh1 affects the level of synaptic-related proteins. Third, conditional Cdh1 knock-out animals may be used to evaluate the effect of Cdh1 on negative emotionality and cognitive deficits induced by tGCI/R directly. However, we still have reason to speculate that the improvement of negative emotion and impaired cognition may be partially attributed to the regulation actions of Cdh1 on hippocampal neuroplasticity after tGCI/R. Find more about the molecules and mechanisms involved in the regulation of emotions and cognition after cerebral ischemia will help us to prevent the generation of negative emotions and cognitive deficits after stroke, thus to improve the prognosis and the quality of life of related patients.

5. Conclusion

In conclusion, this study demonstrate that hippocampal Cdh1

overexpression could improve the negative emotionality and ameliorate cognitive deficits induced by tGCI/R. Simultaneously, Cdh1 overexpression could rescue the loss of hippocampal synapses and dendritic spines, prevent dendritic network disorganization, and enhance neuroplasticity via regulating synaptic and axon growth-associated proteins after ischemia. The insights gained from this work indicate that the application of viral vectors in mechanistic studies is promising and Cdh1 might be a potential therapeutic target for clinical treatment of stroke.

Conflicts of interest

All the authors declared that there have no actual or potential conflicts of interest that could inappropriately influence, or be perceived to influence this work.

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