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Research Paper

Improved memory and reduced anxiety in δ-catenin transgenic mice

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ABSTRACT

δ-Catenin is abundant in the brain and affects its synaptic plasticity. Furthermore, loss of δ-catenin is related to the deficits of learning and memory, mental retardation (cri-du-chat syndrome), and autism. A few studies about δ-catenin deficiency mice were performed. However, the effect of δ-catenin overexpression in the brain has not been investigated as yet. Therefore we generated a δ-catenin overexpressing mouse model. To generate a transgenic mouse model overexpressing δ-catenin in the brain, δ-catenin plasmid having a Thy-1 promotor was microinjected in C57BL/6 mice. Our results showed δ-catenin transgenic mice expressed higher levels of N-cadherin, β-catenin, and p120-catenin than did wild type mice. Furthermore, δ-catenin transgenic mice exhibited better object recognition, better sociability, and lower anxiety than wild type mice. However, both mice groups showed a similar pattern in locomotion tests. Although δ-catenin transgenic mice show similar locomotion, they show improved sociability and reduced anxiety. These characteristics are opposite to the symptoms of autism or mental retardation, which are caused when δ-catenin is deficient. These results suggest that δ-catenin may alleviate symptoms of autism, Alzheimer's disease and mental retardation.

1. Introduction

δ-Catenin is a neural plakophilin-related armadillo protein (NPRAP) (Paffenholz and Franke, 1997). It was first identified by binding with presenilin-1(PS-1) and is abundantly expressed in the brain (Zhou et al., 1997). In addition to PS-1, δ-Catenin binds to E-cadherin, N-cadherin, cortactin, sphingosine kinase, dynamin2, S-SCAM, Kaiso, and many other proteins (Fujita et al., 2004; Ide et al., 1999; Koutras and Levesque, 2011; Lu et al., 1999; Martinez et al., 2003; Rodova et al., 2004). Among these proteins, several, such as N-cadherin, PSD-95, and S-SCAM, are essential to regulate synaptic plasticity (Lu et al., 2002). These proteins are involved in the morphogenesis of dendrite, which is an indicator of synaptic plasticity. Indeed, δ-catenin overexpressing cells affected dendritic morphogenesis (Kim et al., 2008). It is also reported that δ-catenin regulates spine and synapse morphogenesis and functions in hippocampal neurons during development (Arikkath et al., 2009). In addition, δ-catenin is required for maintenance of neural structures and functions in the mature cortex, and δ-catenin mutant mice show deficient learning ability and abnormal synaptic plasticity (Israely et al., 2004; Matter et al., 2009). Furthermore, δ-catenin is mapped to the chromosomal 5p15.2. region; deletion of this region is related to cri-du-chat syndrome, which is severe mental retardation in humans (Medina et al., 2000). On the other hand, patients who have partial deletion of δ-catenin and partial duplication of a δ-catenin promoter showed a mild cri-du-chat syndrome (Sardina et al., 2014). It is also reported that δ-catenin is associated with various neurological disorders, such as autism, schizophrenia, intellectual disturbance, and myopia (Hofmeister et al., 2015; Lam et al., 2008; Lu et al., 2011; Lu et al., 2016; Turner et al., 2015; Vrijenhoek et al., 2008).

Although severe learning deficit and abnormal synaptic plasticity are reported in δ-catenin-deficient mice, the exact role of δ-catenin in brain function and neuronal diseases is not fully understood (Israely et al., 2004). Furthermore, the effect of δ-catenin overexpression in mice has not been investigated yet. In order to identify biochemical and

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behavioral characteristics of δ-catenin overexpression in the brain, we generated δ-catenin transgenic (δTG) mice. Since δ-catenin could induce several types of cancer, like colorectal and prostate cancer (Lu et al., 2005), we decided to specifically express δ-catenin in a mouse brain. We adopted Thy-1 as the promotor for brain-specific expression, and cloned δ-catenin in the Thy-1 promotor vector (Vidal et al., 1990). In this study, we investigated the differences in biochemical and behavioral characteristics between wild type (WT) and δTG mice.

2. Methods

2.1. Animals

Male δTG and WT mice were used in the experiment. The mice were divided into old (9 months, 50 ± 7 g) and young (3 months, 30 ± 5 g) groups (young WT group, $n = 10$; young δTG group, $n = 10$; old WT group, $n = 4$; old δTG group, $n = 10$). Each mouse was housed in a separate cage. Food and water was provided ad libitum to the mice, which were kept under a 12-h light/dark cycle (light was on from 7:00 am to 7:00 pm) at a constant temperature (22 °C) and 55 ± 5% humidity. All animal care and experiments were approved and conducted following the guidelines of the Institutional Animal Care and Use Committee of Chonnam National University Medical School (approval No.: CNU IACUC-H-2014-8).

2.2. Generation of the transgenic mice

To generate δTG mice overexpressing δ-catenin specifically in the brain region, mouse δ-catenin was cloned in a vector with a Thy-1 promoter. The vector having Thy-1 promoter was cut by an xho1 restriction enzyme, and to make a blunt end, DNA polymerase 1, Large (klenow) Fragment was treated, and followed by alkaline phosphate treatment to inhibit self-ligation. At the same time, δ-catenin was acquired from δ-catenin plasmid. Before ligation, the linearized vector and δ-catenin insert were gel-purified by Gel and PCR clean-up kit (#A9281, Promega). Linearized vector and δ-catenin insert were ligated by T4 ligase. After getting plasmid, microinjection into C57b/6 mice was performed by Macrogen (Seoul, Republic of Korea). Xho1 restriction enzyme (#R0146S), DNA polymerase 1, Large (klenow) Fragment (#M0210S), alkalin phosphate (#M0290S), and T4 ligase (#M0202S) were purchased from New England Biolabs. Gel and PCR clean-up kit (#A9281) was bought from Promega.

2.3. Genotyping

A small part of mice tail was cut and dissolved in lysis buffer (100 mM Tris, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 100 μg/ml proteinase K) at 55 °C overnight in a shaking incubator. Phenol (*p*H 7.5–8.0) (#PHE 510.100, Bioshop) was added and rotated for 30 min at room temperature followed by 12,000 rpm centrifuging for 7 min. Then the upper phase was collected. The same step was repeated using phenol/chloroform (#PHE 512.100, Bioshop) and chloroform (#C2432, Sigma) instead of phenol. At the last, the sample was air-dried to evaporate the organic solution. An air-dried sample was used to perform PCR to distinguish between δTG mice and WT mice. Forward primer (ATTCATCATGTGCTCCGTG GATC) binding end of Thy-1 promoter and reverse primer (CTGGCTACGATCTGGCGTTCAGC) binding beginning of the δ-catenin sequence were used for PCR.

2.4. Antibodies

Antibodies used for immunoblotting were commercially available. These were anti-δ-catenin (#611537, BD Bioscience), p120-catenin (#33–9700, ZYMED), β-catenin (#9562, Cell Signaling), N-cadherin (#610920, BD Bioscience), glutamate receptor1 (#8850 Cell Signaling), glutamate receptor2/3/4 (#2460 Cell Signaling), GSK3α/β (#5676 Cell Signaling), phosphor-GSK3α/β (#9331 Cell Signaling), anti-actin (#CP01 Calbiochem).

2.5. Immunoblotting

Tissue was homogenized with MLB lysis buffer. A protein assay was performed to check the concentration of tissue lysates with a BCA kit (#23225, Pierce, Rockford, IL, USA). Same amount of each lysate was loaded onto SDS-polyacrylamide gel for electrophoresis. The separated proteins from the SDS- polyacrylamide gel were transferred to a polyvinylidenedifluoride membrane (#IPVH00010, Millipore, Billerica, MA, USA). The membrane was cut according to the size of the target protein. Membranes were activated by methanol and blocked with 5% skim-milk buffer. Then membranes were treated with a primary antibody and probed with horse-radish peroxidase-conjugated secondary antibodies. Antibody-bound proteins on the membrane were visible with chemiluminescence Western Blotting Detection reagents (#WBKLS0500, Millipore). The density of protein bands was measured by Multi Gauge v3.1 software.

2.6. qPCR

Brain tissues of δTG and WT mice were prepared. Each tissue was homogenized with triazole and subjected to qPCR. RNA (500 ng) from each brain was converted to cDNA by using an M-MLV reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). Quantitative analysis of target and reference genes was performed in triplicate using SYBR green (Enzynomics, Seoul, Korea) on CFX (Bio-Rad, Hercules, CA, USA). Forward primer 5'-AAGGAATGTTAGTTCGGCCGGAGA-3′ and reverse primer 5'-ATCACGTACAGCAAGGCATCCGTA-3′ were used to measure δ-catenin level.

2.7. Behavior tests

2.7.1. Open field test

This test was performed as described (Choi et al., 2017). Mice were placed in an arena (40 × 40 × 40 cm) having white side walls and a grey bottom. The mice were prehabituated in the arena for 5 min. Mice were placed in the center of the arena and freely explored the arena for 20 min. Movement of mice in the arena was recorded and analyzed by the Any-maze system (Stoelting, IL, USA). Total distance and mean speed that mice moved in the arena were measured for locomotion activity. Additionally, freezing time in the open-top arena and time spent in the center of the arena were measured.

2.7.2. Novel object recognition test

This test was done as described (Pan et al., 2012). The novel-object recognition test was performed in the open-top arena (40 × 40 × 40 cm) and consisted of two phases: training and test. During the training phase, each mouse was placed for 10 min in the presence of two identical objects having the same shape and color in each corner of one wall in the arena. After 24 h, one of the two objects was replaced by a novel object, and the mouse was returned to the arena (test phase). Time the mouse spent in each object zone was measured.

2.7.3. Morris water-maze test

This test was performed as described (Wang et al., 2014). A circular pool (diameter: 114 cm, height: 25 cm) was filled with 20 ± 5 °C water. Visual cues were put into the start quadrant and the goal quadrant, which was opposite to the start quadrant. An invisible platform (17 × 10.5 cm) was put in the middle of the target quadrant. Mice started swimming with facing the wall in the quadrant that did not have a platform. The mice were allowed to swim for 40 s. If they found the platform, they were allowed to stay there for 30 s. If the mice failed to find the platform in a limited time, they were guided to the platform.

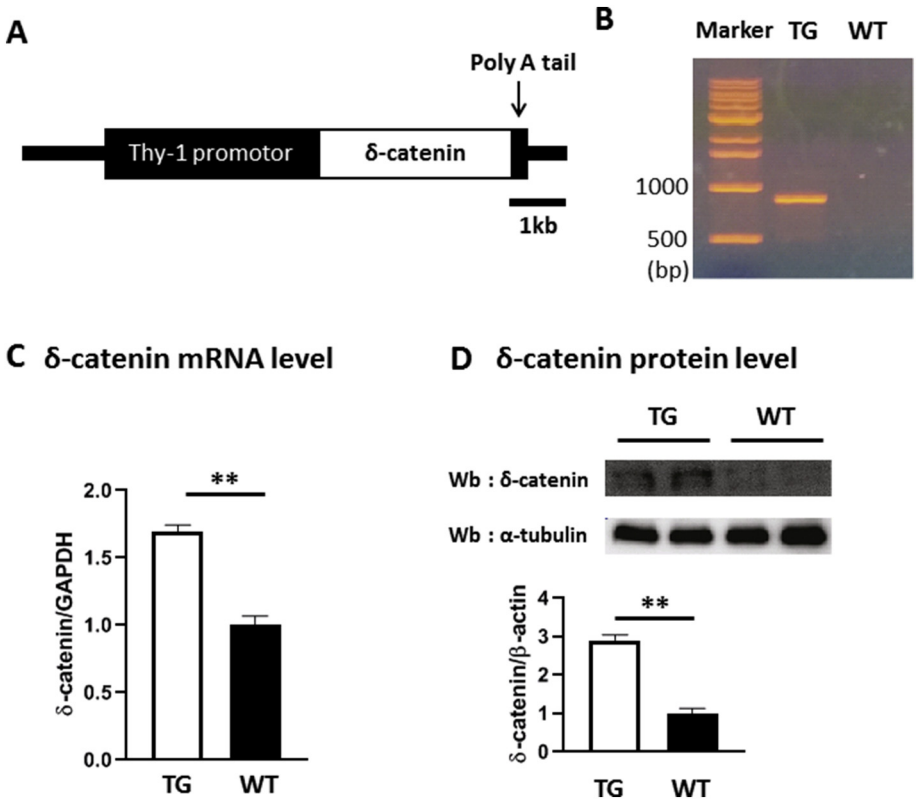

Fig. 1. Generation of the transgenic mice expressing more δ-catenin in the brain. (A) Scheme of construction used to generate δTG mice. δ-Catenin was cloned into vector with a Thy-1 promoter. Polyadenylation sequence was also added at the end of construction. (B) Genotyping of δTG mice and WT mice. PCR was done to distinguish transgenic mice from WT mice. One primer binding end of Thy-1 promoter and the other primer reversely binding to the start site of δ-catenin were used in PCR. (C) Graph shows qPCR data of δTG ($n = 2$) and WT ($n = 2$) mice. mRNA was extracted from half of the brain in transgenic mice and WT mice. δ-Catenin mRNA was amplified, and δ-catenin mRNA expression level in transgenic mice was expressed as a relative value compared to WT mice. (D) Western blot was performed with anti-δ-catenin antibody to check δ-catenin protein levels in WT ($n = 2$) and δTG mice ($n = 2$). α-Tubulin was used as loading control. The density of protein bands was measured by Multi Gauge v3.1 software. $^{**}p < .01$.

Consecutive training for 5 days was performed. A probe test was performed on day 6. Mice were allowed to swim for 60 s without escape-platform in the probe test.

2.7.4. Pole test

This test was done as described (McIlwain et al., 2001). The mice were placed on the top of a vertical wooden rough-surface pole (diameter, 1.5 cm; height, 43 cm) and made to look upward. The time that it took for the mice to turn downward and descend to the bottom was recorded for 60 s. The pole test score was calculated as follows. Fell immediately, 1; fell in 0–10 s, 2; 11–20 s, 3; 21–30 s, 4; 31–40 s, 5; 41–50 s, 6; 51–60 s, 7. Stayed on for 60 s and climbed halfway down, 8. Climbed to lower half of pole, 9. Climbed down and off in 51–60 s, 10; 41–50 s, 11; 31–40 s, 12; 21–30 s, 13; 11–20 s, 14; 1–10 s, 15.

2.7.5. Rota-rod

This test was performed as described (Choi et al., 2017). Mice were placed on a rota-rod and accommodated at 4 rpm for 60 s. During pretraining, mice stayed on the rod with the same speed. The test was performed 3 h later. The rota-rod was accelerated from 4 rpm to 29 rpm over 5 min; 8 trials were performed, and a minimum of 5 min were given between trials. The time to fall was recorded.

2.7.6. Three-chamber test

This test was performed as described (Han et al., 2014). The test apparatus consisted of an acrylic box partitioned into three chambers. The chambers were connected, and the mice could explore every chamber through a hole. During the sociability test, a mouse was placed in one of the lateral chambers, and an empty wire cage was put in opposite lateral chamber. A new wire cage enclosing stranger A was put in the lateral chamber in which the testing mouse was originally placed. The new wire cage and empty wire cage were identical, and stranger A was a novel male mouse that had never encountered the testing mouse. The time that the testing mouse spent exploring the novel mouse or the empty wire cage was analyzed for 10 min. The social novelty test started immediately after the sociability test finished. In the sociability test, the empty cage was replaced by a new cage enclosing stranger B. Stranger B was a male mouse that had also never encountered the testing mouse. The time that the testing mouse spent near each wire cage was analyzed for 10 min.

2.7.7. Elevated plus maze

This test was performed as described (Choi et al., 2017). The maze was arranged as a cross with two open arms (30 cm × 6 cm) facing each other, and two other arms enclosed by high walls (30 cm × 6 cm × 15 cm) with all arms elevated 50 cm from the floor. It was constructed of white plexiglass. A camera was positioned above the maze to record the movements for analysis using automated tracking software (Anymaze, stoeling). Each animal was placed in the center of the maze, facing one of the closed arms. The number of entries into arms and the time spent on the open and closed arms were counted for 5 min.

2.8. Statistical analysis

All data are means ± s.e.m. of at least three independent experiments. Statistical analyses were performed with paired Student's *t*-test. Values of $p > .05$ were considered not significant (n.s). Values of $p < .05$ and $p < .01$ were considered significant and highly significant, respectively.

3. Results

3.1. Generation of the transgenic mice expressing more δ-catenin in the brain

To assess the role of δ-catenin in the brain, we generated a δTG mouse. Mouse δ-catenin was cloned in a Thy-1 promoter expressing vector which included a polyadenylation tail (Fig. 1A). To generate a transgenic mouse overexpressing δ-catenin specifically in the brain, the

plasmid containing δ-catenin under a Thy-1 promoter was micro-injected in C57BL/6 mice. δTG mice were distinguished from WT mice by genotyping their DNAs. Samples from δTG mice showed a PCR band, whereas samples from WT mice did not (Fig. 1B). Furthermore, qPCR was performed with samples obtained from the brain to verify that injected δ-catenin could increase the δ-catenin transcription levels in the brain. As expected, δTG mice showed higher δ-catenin mRNA levels (~1.7-fold increase, Fig. 1C) than the WT mice did. We also compared the δ-catenin protein levels in δTG and WT mice. The δ-catenin protein level was higher in the δTG mice than in the WT mice (Fig. 1D).

3.2. Alteration of δ-catenin-related proteins in transgenic mice

The cadherin-catenin complex is a well-known catenin-protein complex (McMahon and Bradley, 1990). δ-Catenin directly binds to N-cadherin, and this complex affects brain function in a synapse-dependent manner (McMahon and Bradley, 1990). In addition to N-cadherin, GSK3β and glutamate receptors affect brain function and bind to δ-catenin (Farooq et al., 2017; Jones et al., 2002). First of all, we found that the N-cadherin protein level was significantly higher in δTG mice than in WT mice (Fig. 2A). We also checked β-catenin and p120-catenin protein levels, since β-catenin, p120-catenin, and δ-catenin share

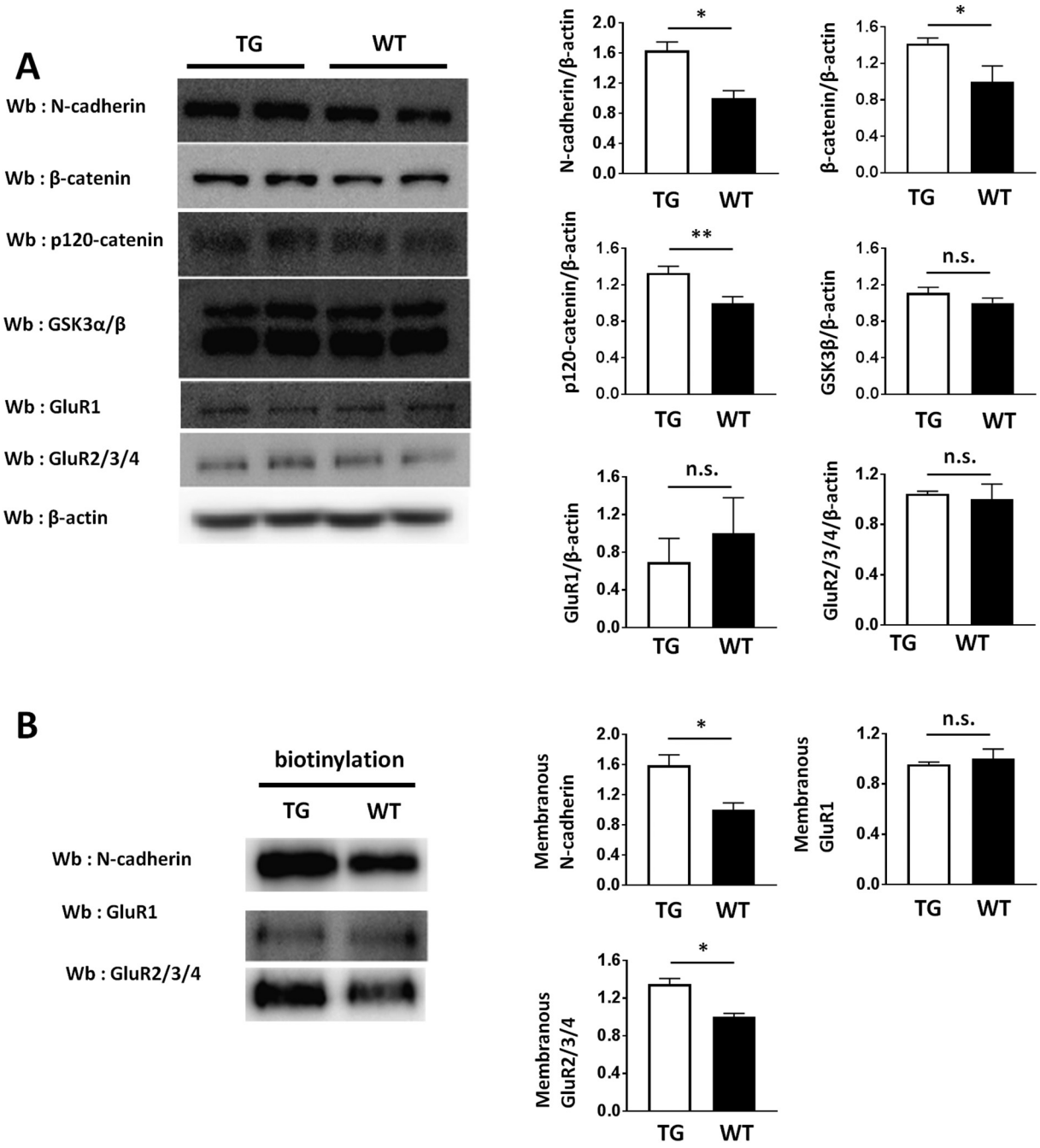

Fig. 2. Alteration of δ-catenin-related proteins in transgenic mice. (A) Total lysates from the brain of δTG mice (n = 2) and WT mice (n = 2) brain were loaded. Western blot was performed to check the N-cadherin, β–catenin, p120-catenin, GSK3α/β, glutamate receptor 1 (GluR1), and glutamate receptor 2/3/4 (GluR2/3/4) with appropriate antibodies. β-Actin was used as a loading control. (D) A biotinylation experiment was performed to check membranous proteins. Membrane levels of N-cadherin, GluR1, and GluR2/3/4 were measured. Each protein level is shown as a bar graph next to western blot data (n = 4). The density of protein bands was measured by Multi Gauge v3.1 software. *p < .05; **p < .01; n.s = not statistically significant.

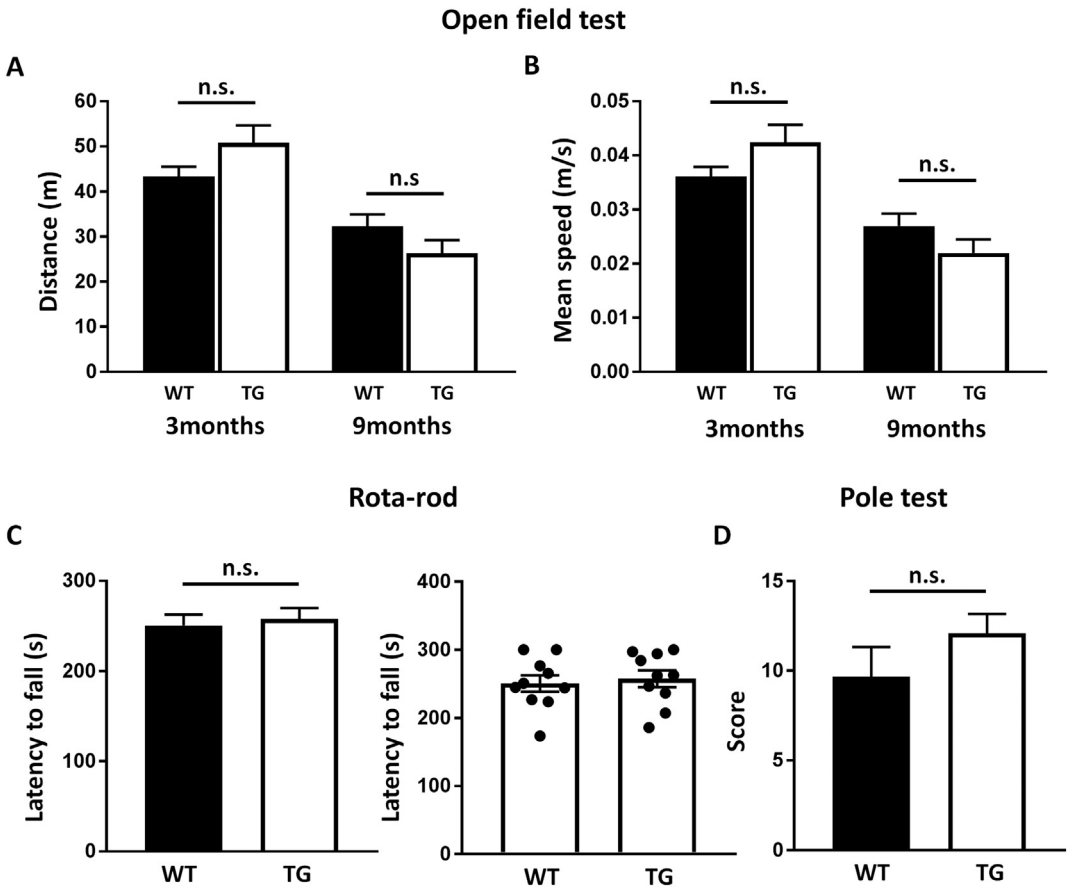

Fig. 3. Locomotion of δTG mice and WT mice. (A) Distance traveled was measured in the open field test (3 months WT, $n = 10$; 3 months δTG, $n = 10$, 9 months WT, $n = 4$, 9 months δTG, $n = 8$). (B) At the same time, mean speed was measured. (C)Latency to fall was measured in the rota-rod test (3 months WT, $n = 10$; 3 months δTG, $n = 10$). (D) Pole-test score was measured in 3-month mice (3 months WT, $n = 10$, 3 months δTG, $n = 10$). n.s = not statistically significant.

GSK3β as a negative regulator. Interestingly, β-catenin and p120-catenin protein levels were increased in δTG mice compared to those in WT mice. Although δ-catenin affected β-catenin and p120-catenin levels, the GSK3β level was not changed (Fig. 2A). We tried to check the effects of δ-catenin on glutamate receptors (GluR). However, no significant change was discovered in the total levels in GluR1 and GluR2/3/4 (Fig. 2A). Furthermore, we checked δ-catenin-related proteins in diverse brain regions (cerebral cortex, hippocampus, cerebellum and brain stem). Because cerebral cortex is the greatest part of brain, protein levels of whole brain were similar to those of cerebral cortex. However, glutamate receptor levels were much higher in hippocampus than in cerebral cortex, cerebellum, and brain stem. In cerebral cortex, the levels of both GluR1 and GluR2/3/4 in the δTG mice were significantly higher than in WT mice, whereas, in the hippocampus, we observed the opposite pattern (Supplementary Fig. 1). Future experiments will be required to clarify the meaning of this discrepancy. Last, we checked membranous proteins by a biotinylation experiment. The membranous N-cadherin level was higher in δTG mice than in WT mice. We also checked the membranous GluR level. Interestingly, though total levels of GluR were not changed, we could detect that the membranous GluR2/3/4 level was high in the δTG mice (Fig. 2B). Shifting of cytoplasmic N-cadherin and GluR2/3/4 to the membrane affects brain function (Jungling et al., 2006; Schrick et al., 2007; Togashi et al., 2002). β-Catenin is related to brain development via Wnt signaling, and p120-catenin is an essential regulator of cadherin (McMahon and Bradley, 1990; Nanes et al., 2012). Since proteins related to brain function were changed in level or localization, behavioral characteristics may be affected.

3.3. Locomotor behavior has no difference in δTG mice and WT mice

In the open field test, young (3 months) and old (9 months) mice moved freely in the arena and their locomotive behavior was measured. We did not find any significant difference between δTG mice and WT mice in the distance traveled (Fig. 3A) and mean speed (Fig. 3B). The young δTG mice showed no significant change on motor activity compared to the young WT mice in the rota-rod test (Fig. 3C). Motor coordination and balance was verified by the pole test, which did not show any difference between the δTG mice and the WT mice (Fig. 3D). Therefore, δ-catenin expression in the brain doesn't affect the movement and balance function of mice.

3.4. δTG mice showed improved object recognition and discrimination

Since δ-catenin binds with presenilin-1, which is closely related to Alzheimer's disease (Jun et al., 2012), we assumed that δTG mice would differ from WT mice in memory function. Mice tend to explore a novel object more than a familiar object spontaneously (Francis et al., 2012). Therefore, the object-recognition test was performed with δTG and WT mice. The test was done with a 24-h retention interval. In it, the δTG mice had spent significantly more time exploring the novel object rather than the familiar object; this was true for both the young mice (Fig. 4A; $p < .05$) and the old mice (Fig. 4B; $p < .05$). Overexpression of δ-catenin in mice brains improved the object recognition memory in the 24-h retention interval test.

Young mice were subjected to the Morris water-maze test to check their spatial memory. The δTG and WT mice learned the location of the escape platform for 5 days, and escape latency in the water pool was

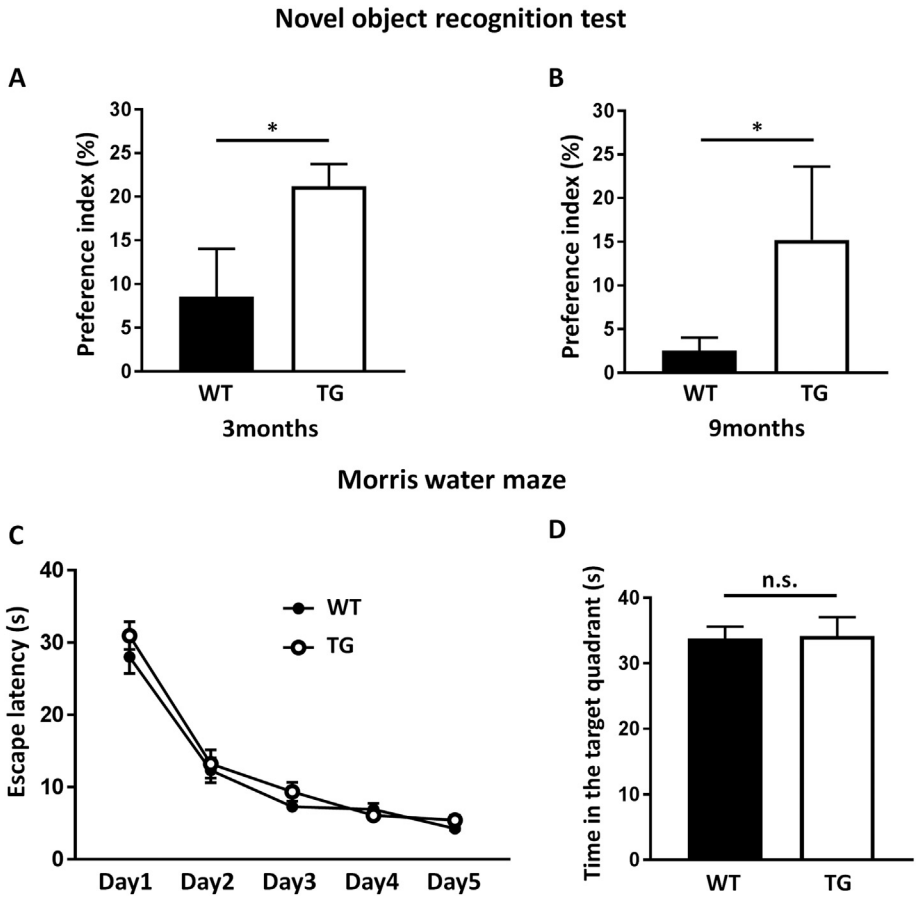

Fig. 4. Memory of δTG mice and WT mice. (A) Novel object recognition test was performed. Novel exploring time that 3-month mice spent on novel object in 24-h retention interval was measured (WT, $n = 10$; δTG, $n = 10$). (B) The same test was performed with 9-month mice (WT, $n = 4$; δTG, $n = 8$). (C) Morris water-maze test was done. Escape latency of δTG mice and WT mice was checked for 5 days. (D) Time that mice spent staying in target quadrant was measured. *$p < .05$; n.s = not statistically significant.

measured. Escape latency was gradually decreased during learning and the curves were not significantly different between δTG and WT mice during the training (Fig. 4C), implying that δTG does not affect learning. In probe test, both groups of mice showed a similar exploring time in the target quadrant in which platform was previously located (Fig. 4D), suggesting that δTG does not affect memory either. Morris water-maze test is a hippocampus-dependent task that assays spatial memory. Thus, spatial memory was not improved by δ-catenin overexpression in this test. These results imply that the expression of δ-catenin contributes to the improvement of cognition and memory of novel object rather than spatial memory.

3.5. δTG mice showed improved social interactions

To measure sociability, the time that mice spent with stranger A or in the empty cage was analyzed. Young δTG mice spent more time exploring stranger A than the empty cage, but not in the young WT mice (Fig. 5A; $p < .01$). Similarly, old δTG mice spent lots of time exploring stranger A rather than the empty cage, but not in the old WT mice (Fig. 5B; $p < .05$). These results suggest that δ-catenin expression improved social interaction activity in transgenic mice.

After the sociability test, a social novelty preference test followed. In the social novelty test, young δTG and WT mice spent about equal time exploring stranger A and stranger B (Fig. 5C). In contrast, old δTG mice spent more time exploring stranger B than stranger A, but the time that WT mice spent with stranger A and with stranger B was not significantly different (Fig. 5D; $p < .01$). These data suggest that social novelty recognition was improved in δTG mice in an age-dependent manner.

3.6. δTG mice showed less anxiety

Anxiety level was tested by the elevated plus maze. In the young group, the δTG mice spent significantly more time in the open arm than the WT mice did, suggesting that the anxiety level was reduced in the δTG mice (Fig. 6A; $p < 0.05$). The anxiety level was also alleviated in old mice group, though the difference of exploring time in the open arm between the δTG mice and the WT mice was not statistically significant (Gap of exploring time; young: 9.46 ± 1.99 sec, old: 5.41 ± 2.07 sec). (Fig. 6B). As shown in Fig. 6B, the anxiety level of the WT mice decreased in the older ones. It is also reported that older animals showed less anxiety in previous studies (Frick et al., 2000; Inta et al., 2013) Interestingly, the open arm time in δTG mice was not increased in old group compared to young one, suggesting the anxiety level was not reduced in old δTG mice (Supplementary table 1). The enhanced cognition and memory might minimize the anxiety level by learning the safety of the open arm quickly in δTG and, age-dependently, gradual decrease of memory and anxiety could compensate each other to stabilize the level of anxiety in the old δTG. Thus, the time gap between WT and δTG might become smaller compared to that of the young mice. Nevertheless, the level of anxiety was generally reduced in δTG compared to WT in these data. Therefore, δ-catenin seems to reduce anxiety.

4. Discussion

In this study, we report biochemical and behavioral characteristics of δTG mice for the first time. Interestingly, the δTG mice showed significantly higher N-cadherin, β-catenin and p120-catenin levels than WT mice, whereas the levels of GluR were not changed significantly. Furthermore, the δTG mice had better object recognition but less anxiety and were more sociable than the WT mice.

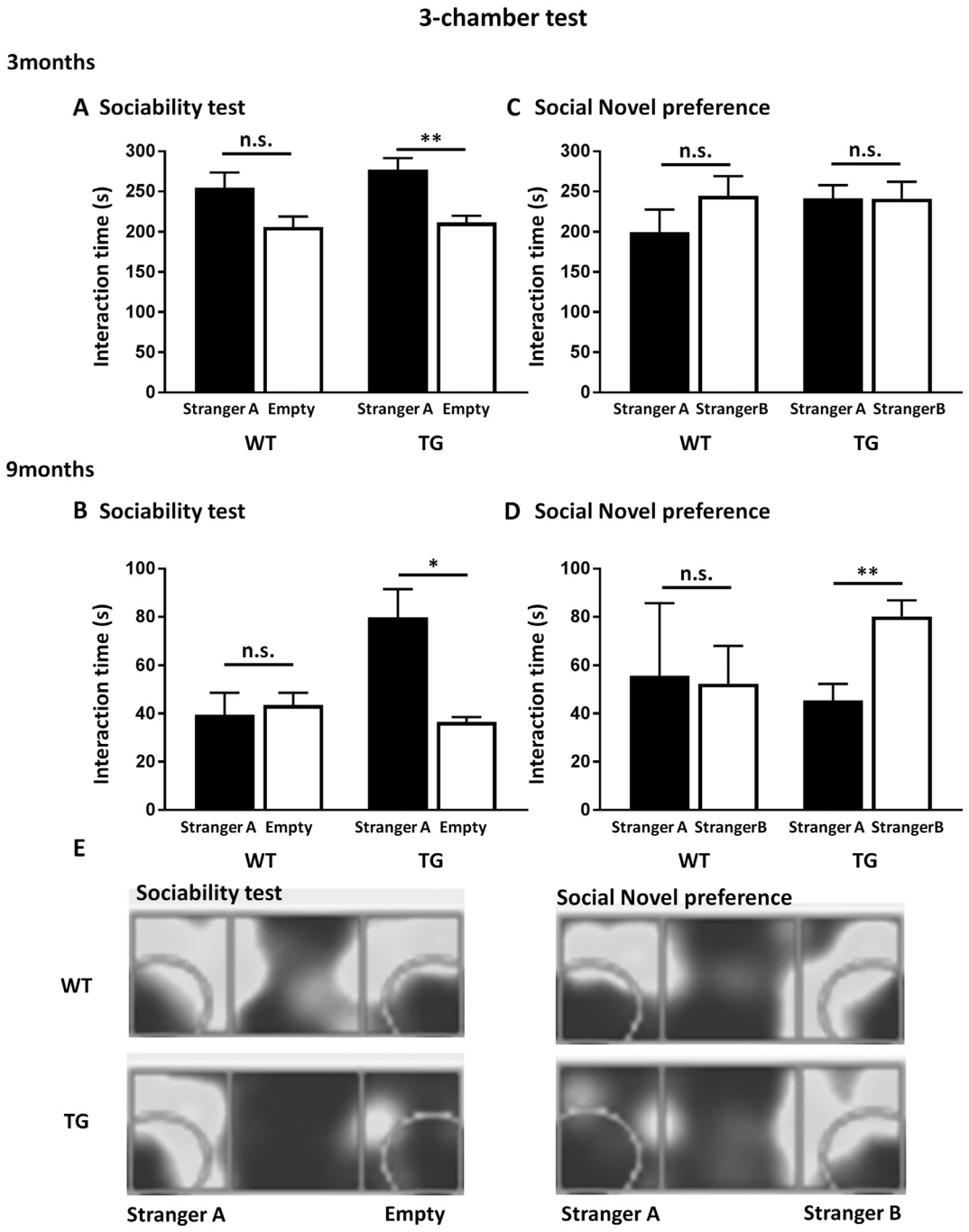

Fig. 5. Sociability of δTG mice and WT mice. (A, C) Sociability test and social novel preference test were performed with 3-month mice (WT, $n = 10$; δTG, $n = 10$). (A) Time mice spent with stranger A and time in empty space were measured in the sociability test. (C) Continuously, stranger B was placed in the previously empty space and social novel preference was measured. (B, D) The same experiments were performed with 9-month mice (WT, $n = 4$; δTG, $n = 8$). (E) Schematic representation of three chamber test. $^{*}p < .05$; $^{**}p < .01$; n.s = not statistically significant.

Cadherin-catenin complexes can modulate synaptic activity (Murase et al., 2002; Tanaka et al., 2000). N-cadherin associated with catenin regulates synaptic strength, synaptogenesis, dendritic spine morphology, synaptic transmission, synaptic transmission, synaptic vesicle dynamics, and activity-dependent synaptic plasticity (Bamji et al., 2006; Chen et al., 2017; Jungling et al., 2006; Murase et al., 2002; Pielarski et al., 2013; Reines et al., 2012; Togashi et al., 2002). N-cadherin protein level is significantly reduced in δ-catenin knockout mice (Israely et al., 2004). Along with these lines, it is of great interest to observe that N-cadherin level is higher in δTG mice than in the WT mice (Fig. 2A).

Binding of p120-catenin to cadherin masks endocytic motifs and stabilizes cadherin (Nanes et al., 2012). Its close relative, δ-Catenin, can bind and stabilize cadherin (Reynolds and Carnahan, 2004). δ-Catenin can be palmitoylated, and palmitoylated δ-catenin stabilizes N-cadherin (Brigidi et al., 2014). δ-Catenin is regulated by a ubiquitin/proteasome pathway via GSK3β phosphorylation (Oh et al., 2009). Since the degradability of GSK3β is not altered, we thought δ-catenin overexpressing causes change in other protein levels. Because δ-catenin is an ARM repeat protein, and it is reported that β-catenin and p120-catenin are substrates of GSK3β, the catenin family may share the same negative regulator (GSK3β). Therefore, β-catenin, p120-catenin and other

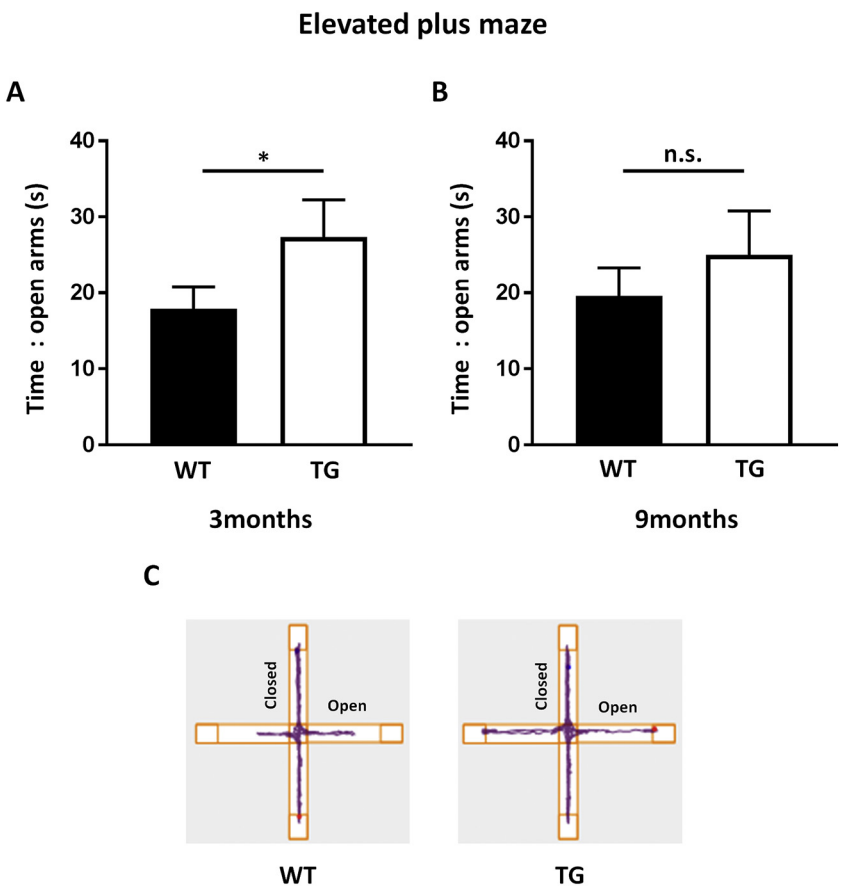

Fig. 6. Anxiety level of δTG mice and WT mice. (A) 3-month mice were placed in the elevated plus maze. The time mice spent in the open arm was measured (WT, $n = 10$; δTG, $n = 10$). (B) The same experiment was done with 9-month mice (WT, $n = 4$; δTG, $n = 8$). (C) Schematic representation of elevated plus maze. *$p < .05$; n.s = not statistically significant.

ARM repeat proteins levels can be altered (Aberle et al., 1997; Xia et al., 2003). As we expected, the β-catenin and p120-catenin level is slightly higher in δTG mice than in WT mice. In conclusion, an increase of cadherin and different kinds of catenin was followed by an increase of the cadherin-catenin complex in δTG mice. This cadherin-catenin increase affects spine formation and dendritic morphogenesis (Murase et al., 2002; Yu and Malenka, 2003). N-cadherin stabilizes newly formed synapses in hippocampal long-term potentiation and is required for memory consolidation (Jungling et al., 2006; Schrick et al., 2007; Tang et al., 1998). So, it was suggested that the increase of N-cadherin might contribute to recognition of objects and formation of memories.

Since δ-catenin can also associate with a glutamate receptor (GluR) in a PDZ-dependent manner (Jones et al., 2002; Ochiishi et al., 2008), we checked GluR level. We couldn't detect a significant change in total protein level. However, membranous GluR2/3/4 is highly expressed in δTG mice, although GluR1 showed no significant difference. δ-Catenin can regulate its interaction with the PDZ binding proteins and GluR2 trafficking to the membrane (Poore et al., 2010). δ-Catenin affects GluR2 via ABP (AMPAR-binding protein) and GRIP (glutamate receptor interaction protein) not PSD95 (Farooq et al., 2017). Although δ-catenin is not associated with GluR1 directly, it has been reported that GluR1/2 heteromerization is responsible for increased membranous GluR1 (Misra et al., 2010; Poore et al., 2010). However, membranous GluR1 is not increased in δTG mice despite the increase of GluR2. Further study would be needed to explain why membranous GluR1 was not altered in this experiment.

Object-recognition memory was improved in the δTG mice. However, memory was not relatively stable in Morris water-maze test (Fig. 4). Previous studies have shown that deletion of δ-catenin causes cognitive dysfunction and abnormal synaptic plasticity (Israely et al., 2004). According to a previous study, the hippocampus and perirhinal cortex are closely related to recognition memory (Hammond et al., 2004; Reger et al., 2009). Furthermore, δ-catenin regulates spine and synapse morphogenesis in hippocampal neurons (Kourtidis et al., 2013). These studies suggest that δ-catenin may contribute to learning and object-recognition memory.

The δTG mice showed more social interaction than the WT mice (Fig. 5). Furthermore, the δTG mice showed less anxiety-like behavior in an elevated plus maze. One may argue that such behavior might have resulted from general elevation of motor activity. However, locomotion did not seem to affect other results (Fig. 3). The δTG mice would have less anxiety (Fig. 6). δ-Catenin is a critical gene in autism and an important neurodevelopmental protein as well (Medina et al., 2000; Turner et al., 2015). δ-Catenin correlates with many autism genes and affects autism through various mechanisms (Turner et al., 2015). δ-Catenin binds to cortactin, and the δ-catenin–cortactin complex can regulate neuronal morphogenesis (Martinez et al., 2003). Cortactin binding protein2 controls cortactin-related dendritic spinogenesis and is known as an autism gene correlated with δ-catenin (Chen et al., 2012; Cheung et al., 2001; Turner et al., 2015). Shank is known as an autism gene and antagonizes dendritic branching induced by Densin-180 by competing with δ-catenin (Quitsch et al., 2005). Other research groups found that genetic alteration of δ-catenin leads to neurological disorders such as Cri-du-chat syndrome, Alzheimer's disease, autism, and schizophrenia (Hofmeister et al., 2015; Jun et al., 2012; Lam et al., 2008; Lu et al., 2011; Lu et al., 2016; Sardina et al., 2014; Turner et al., 2015; Vrijenhoek et al., 2008). Accordingly, δTG mice had better sociability and less anxiety than the WT mice. Raising the δ-catenin level may need to be further studied as a therapeutic treatment for sociability deficiency and high anxiety.

5. Conclusion

We generated δTG mouse to investigate role of δ-catenin in mouse

brain. Alteration of N-cadherin and GluR was investigated in δTG mice. β-Catenin and p120-catenin protein levels were changed in δTG mice as well. These biochemical alterations changed behavioral characteristics of the δTG mice. Object recognition was improved, as was social interaction. In addition, anxiety was reduced. We described different characteristics of δTG mice compared to those of WT mice. Understanding how δ-catenin works in the brain may lead to discovery of potential treatments for various mental illnesses including autism, Alzheimer's disease and mental retardation.

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