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Targeting β-Catenin in GLAST-Expressing Cells: Impact on Anxiety and Depression-Related Behavior and Hippocampal Proliferation

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Abstract

β-catenin (key mediator in the Wnt signaling pathway) contributes to the pathophysiology of mood disorders, associated to neurogenesis and neuroplasticity. Decreased β-catenin protein levels have been observed in the hippocampus and prefrontal cortex of depressed subjects. Additionally, the antidepressants exert, at least in part, their neurogenic effects by increasing β-catenin levels in the subgranular zone of the hippocampus. To further understand the role of β-catenin in depression and anxiety, we generated two conditional transgenic mice in which β-catenin was either inactivated or stabilized in cells expressing CreERT under the control of the astrocyte-specific glutamate transporter (GLAST) promoter inducible by tamoxifen, which presents high expression levels on the subgranular zone of the hippocampus. Here, we show that β-catenin inactivation in GLAST-expressing cells enhanced anxious/depressive-like responses. These behavioral changes were associated with impaired hippocampal proliferation and markers of immature neurons as doublecortin. On the other hand, β-catenin stabilization induced an anxiolytic-like effect in the novelty suppressed feeding test and tended to ameliorate depressive-related behaviors. In these mice, the control over the Wnt/β-catenin pathway seems to be tighter as evidenced by the lack of changes in some proliferation markers. Moreover, animals with stabilized β-catenin showed resilience to some anxious/depressive manifestations when subjected to the corticosterone model of depression. Our findings demonstrate that β-catenin present in GLAST-expressing cells plays a critical role in the development of anxious/depressive-like behaviors and resilience, which parallels its regulatory function on hippocampal proliferation. Further studies need to be done to clarify the importance of these changes in other brain areas also implicated in the neurobiology of anxiety and depressive disorders.

Keywords β-catenin · Depressive-like behavior · Anxious-like behavior · Hippocampal proliferation · Depression animal model

Rebeca Vidal and Emilio Garro-Martínez contributed equally to this work.

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Introduction

β-catenin (Armadillo in Drosophila) is a conserved molecule that exerts a crucial role in a multitude of developmental and homeostatic processes. More specifically, β-catenin has a dual role as the key nuclear effector of canonical Wnt signaling in the nucleus [1], and as an integral structural component of the cadherin-based adherent junctions [2]. β-catenin is maintained at low levels through GSK-3β-mediated phosphorylation. After GSK-3β inhibition, β-catenin is accumulated in the cytoplasm and translocated to the nucleus where it activates target genes by binding to TCF/LEF factors [3, 4]. β-catenin also plays an important role in cell adhesion, enhancing dendritic arborization as well as synaptic plasticity in hippocampal adult neurons [5].

Studies in postmortem human brain samples of suicide victims suffering of major depressive disorder (MDD) show a decrease in β-catenin expression levels both in prefrontal cortex (PFCx) and hippocampus (Hp) [6–8]. Additionally, several studies highlight the involvement of the Wnt/β-catenin pathway in the mechanism of action of antidepressant treatments, since β-catenin expression is increased in the subgranular zone (SGZ) of the hippocampus after electroconvulsive shock (ECS) [9], chronic venlafaxine treatment [10], drugs with a fast onset of antidepressant action as the 5-HT_4 partial agonists [11], the association of SSRIs with a 5-HT_{2A} receptor antagonist [12], or mood stabilizers as lithium [13].

It is widely accepted that adult-born neurons in the dentate gyrus contribute to the efficacy of antidepressants [14–17]. However, several studies indicate that adult neurogenesis is not a principal mediator of the behavioral actions of antidepressants [18, 19], adding certain controversy to the field. Conflicting findings and hypotheses may reflect heterogeneity of experimental design, as the technical approaches used to block the generation of new neurons (gamma or ionizing radiation) are far from being selective methods to avoid adult neurogenesis, whereas conditional gene deletion has been previously employed as selective adult neurogenesis ablation [15, 20]. The use of genetic technology can shed light on the implication of adult hippocampal neurogenesis in the pathophysiology of anxiety and depression, and on the role of the new neurons in the antidepressant mechanism of action. Whether adult neurogenesis is involved in the modulation of anxiety- and depression-related manifestations or whether the increase in adult hippocampal neurogenesis is sufficient to improve mood is still unclear. The changes on β-catenin levels in the hippocampus following chronic antidepressant treatment are associated to an increase in hippocampal cell proliferation. Indeed, the expression of β-catenin in the newborn cells of the SGZ is enhanced following antidepressant treatment [9–11]. Previous studies describe the implication of β-catenin in the control of the balance between neural progenitor expansion and differentiation during the brain development [21, 22]. However, little is known about the role of β-catenin in the generation of new neurons in the adult hippocampus and its behavioral implications. In the last years, it has been described the important role of the Wnt/β-catenin pathway in this neurogenic process [23]. However, further studies are needed to elucidate causal relationships between β-catenin, neurogenesis, and behavior under pathological conditions [23].

In this work, we have generated a place and time conditional mouse line with β-catenin inactivated (cKO) or stabilized (cST) in glutamate aspartate transporter (GLAST)-expressing cells to study the implication of β-catenin in adult hippocampal proliferation, and consequently in the manifestations of anxiety/depression-related behaviors. We have also subjected the conditional mouse stabilizing β-catenin to chronic corticosterone administration in order to determine the influence of β-catenin in the resilience to this animal model of depression.

Materials and Methods

Animals

Male mice (2–3 month old, 25–30 g) were group-housed with 12-h light-dark cycle. Food and water were given *ad libitum* unless otherwise stated. All procedures were carried out with the previous approval of the Animal Care Committee of the University of Cantabria and according to the Spanish legislation and the European Communities Council Directive on "Protection of Animals Used in Experimental and Other Scientific Purposes" (86/609/EEC).

Generation of Transgenic Mice

The GLAST-Cre recombinase transgenic mice maintained on a C57BL6/J background were obtained from Dr. Magdalena Götz [24]. Mice with floxed β-catenin gene (*Ctnnb1*$^{flox/flox}$) maintained on a C57BL6/J background were obtained from The Jackson Laboratory (USA). These mice possess loxP sites located in introns 1 and 6 of the β-catenin gene. GLAST-Cre mice mated with this mouse line generate animals that inactivate β-catenin in progenitor cells of the hippocampus (cKO mice).

Mice stabilizing β-catenin (*Ctnnb1*$^{(ex3)Fl/Fl}$) maintained on a 129/Sv background [25] were generously provided by Prof. M. Mark Taketo. These mice have the Ctnnb1 exon 3 flanked by loxP sites [25]. GLAST-Cre-induced recombination leads to removal of exon 3 (containing the GSK3β phosphorylation sites), and the stabilization of β-catenin protein (cST mice). GLAST-Cre mice were crossbred with *Ctnnb1*$^{flox/flox}$ or *Ctnnb1*$^{(ex3)Fl/Fl}$ mice to obtain GLAST-Cre/*Ctnnb1*$^{flox/flox}$ (cKO) and GLAST-Cre/*Ctnnb1*$^{(ex3)Fl/Fl}$ (cST) mice, respectively. Littermate mice carrying no GLAST-Cre (*Ctnnb1*$^{flox/}$

flox and *Ctnnb1*$^{(ex3)Fl/Fl}$ mice (for cKO and cST, respectively) were used as controls (WT animals).

Mice carrying the R26R LacZ reporter allele maintained on a C57BL6/J background were used to monitor the efficiency of inducible recombination of Cre recombinase in the brain of GLAST-Cre mice [26].

Experimental Design

cKO/cST Phenotype Induction

cKO and cST animals were administered tamoxifen or vehicle (corn oil) (1 mg/day administered in two daily injections for 5 days, i.p.). One month later, they were used for immunohistochemical analyses (Fig. 1a, set 1) or subjected to a battery of behavioral tests (Fig. 1a, set 2).

Chronic Corticosterone Model in cST Animals

A different set of cST and WT animals treated with tamoxifen was subjected to the corticosterone depression model. After 4 weeks of tamoxifen administration, mice were chronically administered corticosterone in the drinking water (5–10-mg/kg/day dose range) during four additional weeks [27]. Then, they were tested in different behavioral paradigms (Fig. 1b).

Behavioral Assays (See Supplemental Information)

Behavioral tests were ordered from the least to most stressful one in consecutive days (open-field, light-dark box, novelty suppressed feeding, sucrose preference, and forced swimming tests) (Fig. 1). Tests were performed during the light phase. Animals were transported to the experimental room 1 h before each experiment to acclimatize.

Open-Field Test The open-field apparatus was a brightly lit (350 lx) white wooden box (50 × 50 × 30 cm) with white floor and bright walls. Mice were released in the center of the apparatus for 5 min, and the total distance traveled was video-tracked by a computerized system (Any-maze Video-Tracking software, Stoelting Co., USA).

Light/Dark Box Test This test was performed in an arena formed by two equally sized compartments (15 × 30 × 20 cm) separated by an opening at the floor level (6 × 6 cm). The lit compartment was brightly illuminated (400 lx). Mice were placed in one corner of the light compartment. Animal behavior was recorded during a 5-min testing session. The anxiety level was represented as the ratio of the time spent in the light vs. the dark compartment.

Novelty Suppressed Feeding Test Mice were food deprived 24 h before testing. The apparatus floor (50 × 50 × 30 cm)

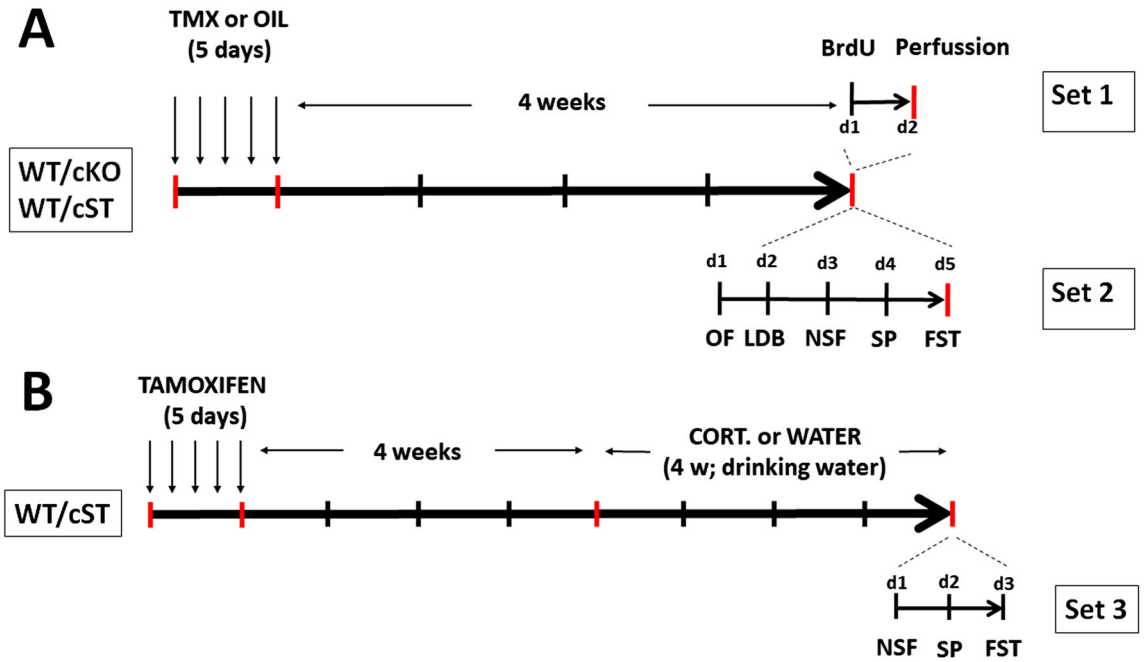


Fig. 1 Experimental schedule of the different approaches followed in this study. Tamoxifen or vehicle (oil) was administered during 5 days, and the neurochemical and behavioral phenotype of mice with β-catenin inactivated or stabilized in GLAST-Cre expressing cells was measured 4 weeks later (**a**). The chronic corticosterone (CORT) model of depression was established in mice with stabilized β-catenin, 4 weeks after the administration of tamoxifen. After 4 weeks of corticosterone treatment, a series of behavioral tests were performed in these animals in consecutive days (**b**). *TMX* tamoxifen, *OF* open-field test, *LDB* light/dark box, *NSF* novelty suppressed feeding, *SP* sucrose preference, *FST* forced swimming test, *WT* wild type, *cKO* β-catenin inactivated, *cST* β-catenin stabilized

was covered with wood-chip bedding, and the light intensity was 30–50 lx. A food pellet was placed in the center of the arena. The time to approach and eat a pellet located in the center of a brightly illuminated arena was recorded during 10 min. After the test, each mouse was transferred to its home cage and the amount of food consumed during a 5-min period was measured. The animals that did not eat during the novelty suppressed feeding (NSF) test session were assigned a latency value of 10 min. Those animals showing no food intake in their home cage were excluded from the data analysis. Survival analysis and statistical differences between the latencies were determined using the Kaplan–Meier product-limit method.

Sucrose Preference Test Mice were individualized and given a free choice between a 1% sucrose solution or water for 24 h. The sucrose preference was calculated as a percentage of the consumed sucrose solution compared to the total amount of liquid intake.

Forced Swimming Test Mice were placed in cylinder tanks (30 × 20 cm) filled with water at 25 °C for a 6-min session, and the last 4 min were recorded. The time spent immobile, swimming, and climbing was scored by a blind observer to the experimental group [28]. The immobility time was divided in 2-min intervals.

Immunohistochemistry

Animals were anesthetized and transcardially perfused with 4% paraformaldehyde. Brains were post-fixed for 4 h at 4 °C, and cryoprotected with 30% sucrose in PBS. Forty-micrometer-thick free-floating coronal brain sections were processed for immunohistochemical experiments.

β-Catenin Immunohistochemistry This assay was performed using a previously described protocol [10], using a rabbit anti-β-catenin antibody (1:500; abcam plc., UK). β-catenin-positive cells were labeled using diaminobenzidine (DAB) + Ni as chromogen (Vector Laboratories).

Ki-67 Immunohistochemistry Sections were incubated with rabbit anti-Ki-67 antibody (1:500, abcam plc., UK). Ki-67$^+$ cells were labeled using diaminobenzidine (DAB) + Ni as chromogen (Vector Laboratories, USA).

BrdU Immunohistochemistry This protocol was performed as previously described [10], using a monoclonal mouse anti-BrdU antibody (1:600; Roche Diagnostics, Spain), and developed using diaminobenzidine (DAB) + Ni as chromogen (Vector Laboratories, USA).

DCX Immunohistochemistry Sections were incubated with goat polyclonal anti-DCX antibody (1:200; Santa Cruz Biotechnology Inc., USA), and the signal was developed using a DAB + Ni solution.

One of every six sections throughout the hippocampus was processed and counted under a bright-field microscope (Carl Zeiss Axioskop 2 Plus) at ×40 magnification and 0.65 numerical aperture (NA) of the objective lenses. The average number of BrdU$^+$, Ki-67$^+$, or DCX$^+$ cells *per* section (six to eight sections) *per* animal was determined.

Ki-67/BrdU Immunofluorescence Detection of Ki-67 in combination with BrdU was performed using an immunofluorescence labeling protocol as previously described in detail [29]. Fluorescent signals were detected using a Zeiss Axio Imager M1 fluorescence microscope, 12-bit B&W camera (AxioCam MRm), cubes: GFP (E_x 470/40–E_m 525/50) and rhodamine (E_x 546/12–E_m 608/65) and objective: ×40/NA 0.75.

Sox2 and GFAP Immunofluorescence Sections were incubated with the primary antibodies rabbit anti-GFAP (1:1000, Dako, Spain) and goat anti-Sox2 (1:1000, Merk, Germany), and the secondary antibodies donkey anti-rabbit Alexa Fluor 488 and donkey anti-goat Alexa Fluor 568 (Invitrogen, USA). Confocal images (1024 × 1024 pixels; 0.179-mm pixel size) were acquired sequentially on a SP5 laser-scan microscope (Leica) with a 63 × 1.4 NA objective. For total cell counts, labeled cells were counted in every six sections through the entire rostrocaudal length of the DG. To count NSCs, cells were deemed radial if the cell body clearly associated with a DAPI-positive nucleus that was located in the subgranular zone and had a single GFAP+ radial process extending through the granule layer, and were positive for Sox2.

LacZ/GFAP Staining The X-gal staining was performed following a previously described protocol [24], followed by the incubation with the primary antibody rabbit anti-GFAP (1:500, Dako Spain). GFAP$^+$ cells were labeled using diaminobenzidine (DAB) + Ni as chromogen (Vector Laboratories, USA) (see Supplemental Information).

Analysis and Statistics

Results are expressed as mean ± standard error of mean (S.E.M.). The statistical analysis of the results was performed using Student's *t* test (for cKO or cST and their respective WT animals treated with tamoxifen), or two-way ANOVA followed by Student-Newman-Keuls post hoc test. Graphs and statistical analyses were done using the GraphPad Prism software, version 6.1 (GraphPad Software Inc., USA). The level of significance was set at $p < 0.05$. The number of animals used in each experimental group is indicated in the "Results" section and figure legends. Complete statistical analyses are summarized in Supplementary Table S1.

Results

β-Catenin Inactivation or Stabilization Localizes Mainly in the Subgranular Zone of the Hippocampus

The role of β-catenin was studied inactivating (cKO) or stabilizing (cST) β-catenin by crossing *Ctnnb1*$^{flox/flox}$ mice (cKO) [30], or *Ctnnb1*$^{(ex3)Fl/Fl}$ mice (cST) [25] with GLAST-CreERT2 mice [24, 31]. The recombination efficiency after tamoxifen induction was checked crossing the GLAST-CreERT2 line with the ROSA26 LacZ reporter line. The colocalization of the X-gal staining with GFAP immunolabeling revealed an efficient and specific recombinase activity in neural stem cells and astrocytes present in the SGZ of the dentate gyrus of the hippocampus (52%), with low signal in astrocytes present in the hilus (6%) and the molecular layer (10%) of the dentate gyrus, and in cortical regions (8%) (Supplementary Fig. S1A–C).

Tamoxifen induction reduced the characteristic β-catenin accumulation in the SGZ of the hippocampus in the β-catenin inactivated mice group ($p < 0.05$) (Fig. 2a, d, e; Supplementary Fig. S2A). In the β-catenin stabilized animals, we did not appreciate changes in the number of β-catenin$^+$ cells in the SGZ of the hippocampus 1 month after tamoxifen administration (Fig. 2b; Supplementary Fig. S2B), but a trend was observed 24 h after transgenic induction (Fig. 2c; Supplementary Fig. S2C). No differences were observed in the β-catenin cKO and cST animals and their respective WT treated with oil, compared to the WT tamoxifen group.

β-Catenin in GLAST-Expressing Cells Modulates Anxiety-like Behavior

We studied the behavioral implication of the elimination or the stabilization of β-catenin in GLAST-expressing cells, comprising a high expression in the SGZ of the dentate gyrus of the hippocampus, in different anxiety-related paradigms. Behavioral values differed between wild-type animals for both cKO and cST groups, since their strain background was different [32]. In the light/dark box (LDB) test, cKO mice showed increased anxiety-like behavior as evidenced by the lower time spent in the lit area compared to the time in the dark side ($p < 0.01$) (Fig. 3a; Supplementary Fig. S3A). The β-catenin cST mice showed no changes compared to their WT mice counterparts (Fig. 3d; Supplementary Fig. S3D). The distance traveled and the number of transitions were similar in the different experimental groups (Fig. 3b, c, e, f; Supplementary Fig. S3B, C, E, F).

No differences were observed between the transgenic mice and their WT counterparts in the locomotor activity evaluated by the total distance traveled in an open-field arena (Fig. 3g, h; Supplementary Fig. S3G, H).

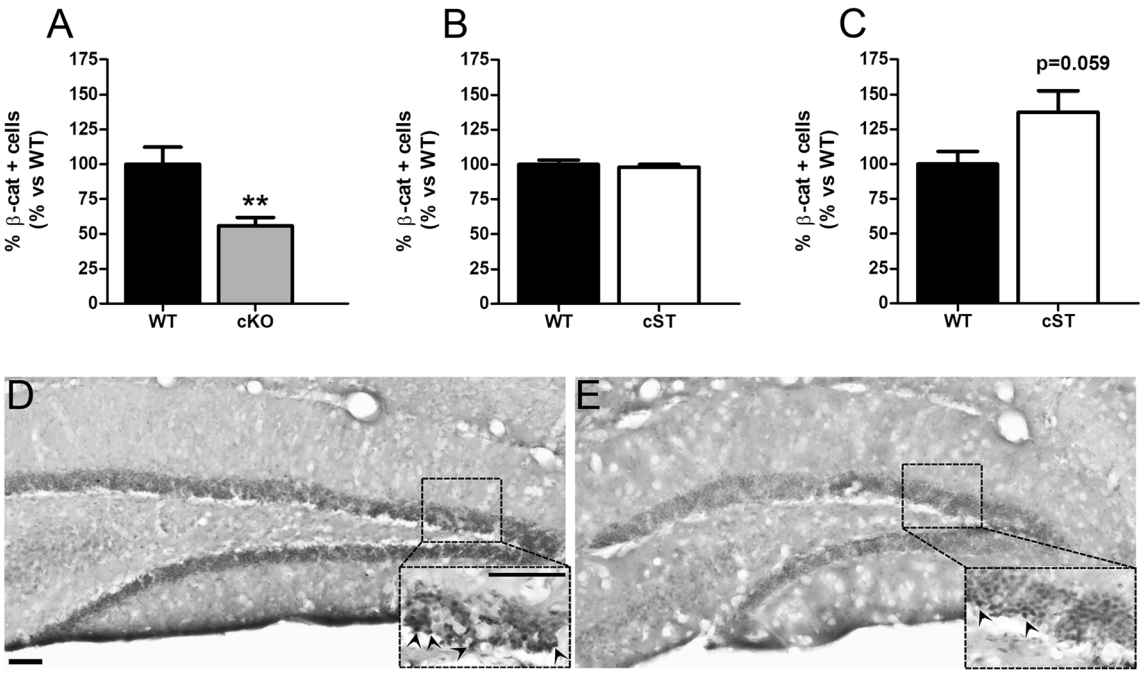


Fig. 2 Graphs showing β-catenin$^+$ cells in the different experimental groups. WT and cKO animals 1 month after the administration of tamoxifen (**a**). WT and cST animals 1 month after tamoxifen administration (**b**). 24 h post-TMX administration (**c**). Representative images of β-catenin immunolabeling in the DG of the hippocampus in WT and cKO mice (**d**, **e**, respectively). Insert: black arrowheads mark β-catenin positive cells. Scale bars: 50 μm. Data are expressed as mean ± SEM. The statistical analysis was performed using an unpaired Student's *t* test; $^{**}p < 0.01$. $n = 6–7$ animals *per* group

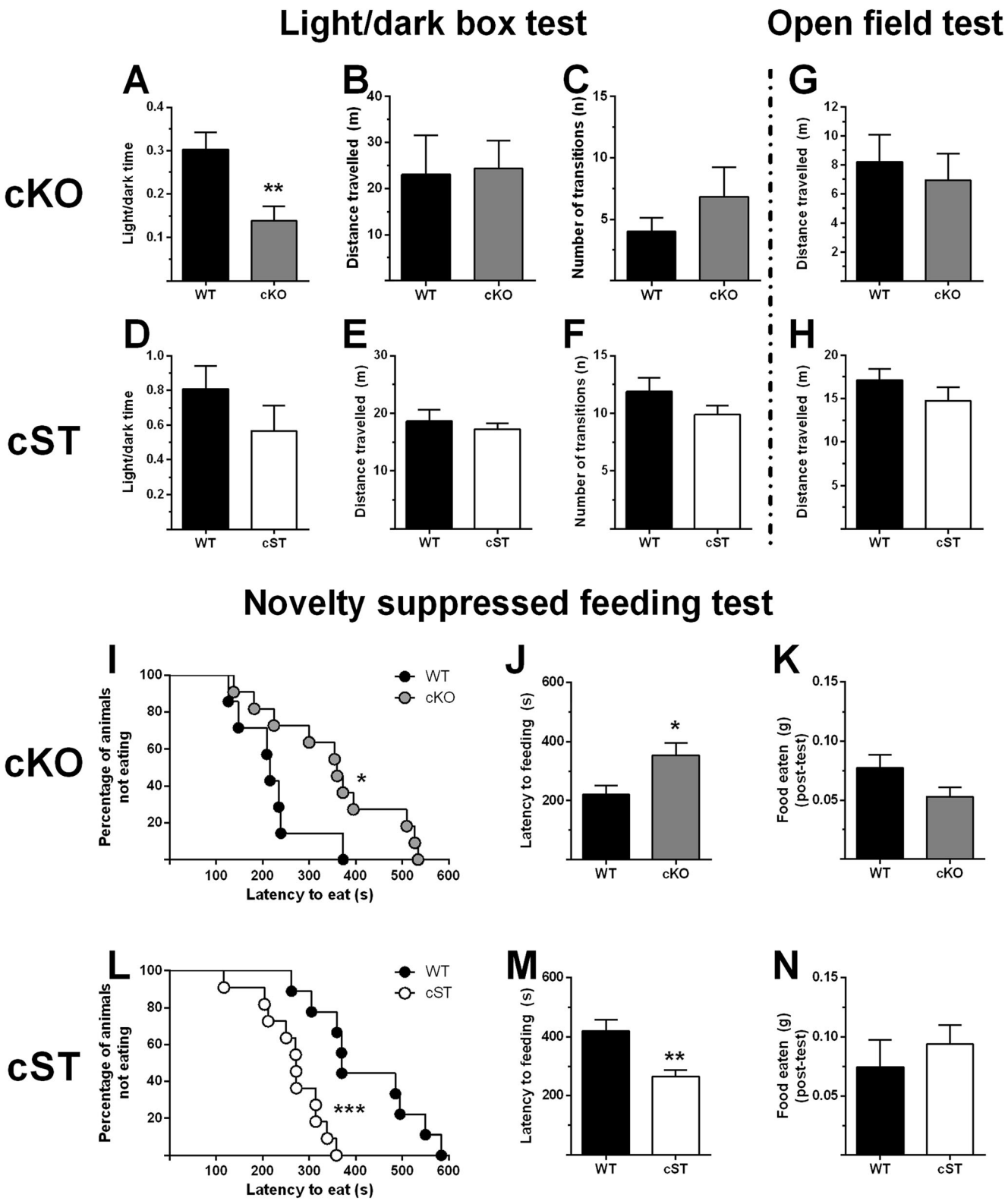


Fig. 3 Evaluation of anxiety-like behavior in mice with β-catenin inactivated (cKO) (**a**–**c**, **g**, **i**–**k**) or stabilized (cST) (**d**–**f**, **h**, **l**–**n**) and their respective WT counterparts. In the light/dark box test, time in the lit compartment versus time in the dark compartment in the LDB test (**a**, **d**), distance traveled (**b**, **e**), and number of transitions (**c**, **f**). In the open-field test, the distance traveled (**g**, **h**). In the novelty suppressed feeding test, latency (s) to approach and eat the pellet (**i**, **j**, **l**, **m**), and food eaten during the post-test (**k**, **n**). Data expressed as the mean ± SEM. Student's *t* test; $*p < 0.05$, $**p < 0.01$. Survival analysis and statistical differences between the latencies in the NSF test determined by the Kaplan–Meier product-limit method (**g**, **j**); $*p < 0.05$ and $***p < 0.001$ vs. WT animals. $n = 7$–11 animals *per* group

In the NSF test, β-catenin cKO mice showed a significant increase in the latency of feeding compared to WT mice ($p < 0.05$) (Fig. 3i, j; Supplementary Fig. S3I, J). On the other hand, β-catenin cST mice elicited a decrease in the latency of feeding compared to WT animals ($p < 0.01$) (Fig. 3l, m; Supplementary Fig. S3L, M). There were no differences in home food consumption between WT and cKO or cST mice (Fig. 3k, n; Supplementary Fig. S3K, N).

β-Catenin in GLAST-Expressing Cells Modulates Depression-Related Behavior

The sucrose preference in the β-catenin cKO animals was lower compared with their wild-type counterparts ($p < 0.01$) (Fig. 4a; Supplementary Fig. S4A), indicative of an anhedonic state. The β-catenin cST animals showed no changes in sucrose preference compared with the WT group (Fig. 4c; Supplementary Fig. S4E).

In the forced swimming test (FST) that measures the behavioral despair, the β-catenin cKO animals presented higher immobility during the final 2 min of the test compared to their wild-type counterparts ($p < 0.05$), and an increase in immobility over time ($p < 0.01$) (Fig. 4b; Supplementary Fig. S4B), although no changes were observed in the swimming and climbing behavior (Supplementary Fig. S4C, D, respectively). The β-catenin cST mice showed no changes in the immobility time compared to WT mice (Fig. 4d; Supplementary Fig. S4F), and in the swimming time (Supplementary Fig. S4G).

β-Catenin Modulation of Hippocampal Proliferation in the Adult Brain

The number of BrdU-immunoreactive cells in the SGZ of the hippocampus of β-catenin cKO mice was significantly lower compared with their WT littermates ($p < 0.001$) (Fig. 5a, b, g; Supplementary Fig. S5A). A decrease in the number of Ki-67$^+$ cells was also observed ($p < 0.001$) (Fig. 5c, d, h; Supplementary Fig. S5B). Moreover, the deletion of β-catenin in the GLAST-expressing cells decreased the number of DCX-positive cells in the SGZ of the dentate gyrus of the hippocampus compared with their WT counterparts ($p < 0.01$) (Fig. 5e, f, i), with no changes in the number of dendritic intersections (Fig. 5j).

On the other hand, the stabilization of β-catenin in the cST animals induced an increase in Ki67$^+$ cells ($p < 0.05$) (Fig. 5l; Supplementary Fig. S5D), while no changes were observed in the number of BrdU$^+$ or DCX$^+$ cells 1 month after tamoxifen administration (Fig. 5k, m, respectively; Supplementary Fig. S5C). The analysis of the dendritic intersections did not show differences compared to their wild-type counterparts (Fig. 5n).

To study the neural progenitor cell cycle progression, we performed a colocalization of BrdU and Ki67 labeling (Fig. 6b–d). The cell cycle reentry, evaluated by the ratio BrdU$^+$Ki67$^+$/BrdU$^+$, presented a significant increase in cST mice ($p < 0.001$) (Fig. 6a; Supplementary Fig. S6).

Conditional ablation of β-catenin resulted in a significant decrease in the number of neural progenitor cells in the SGZ

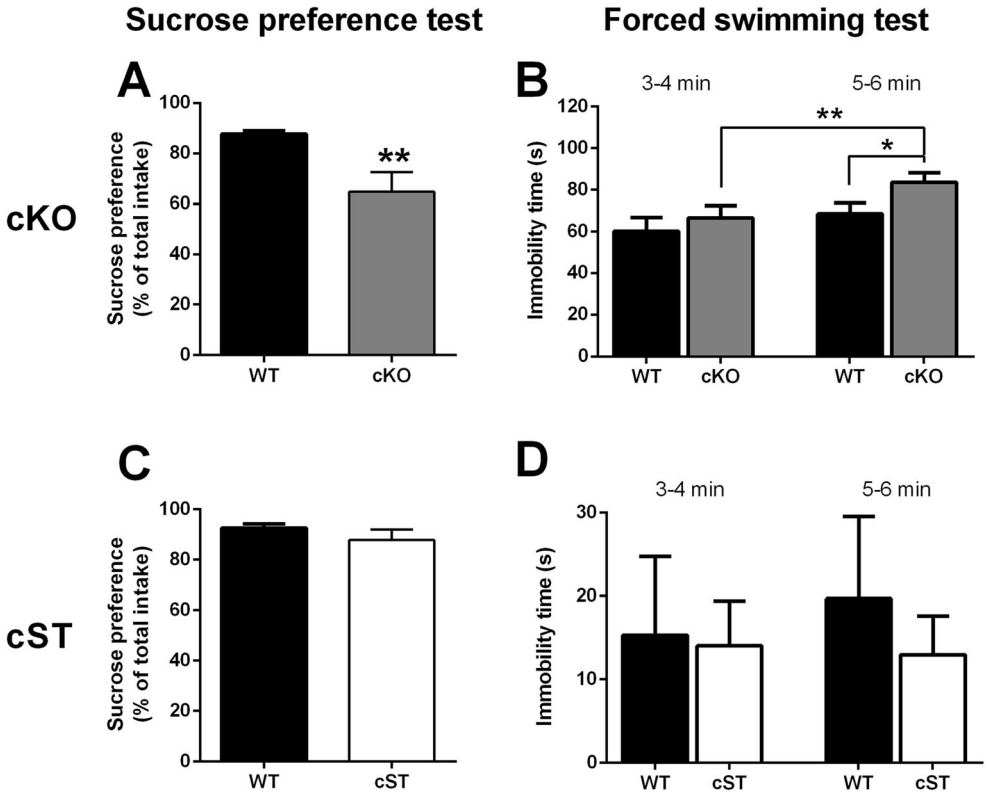
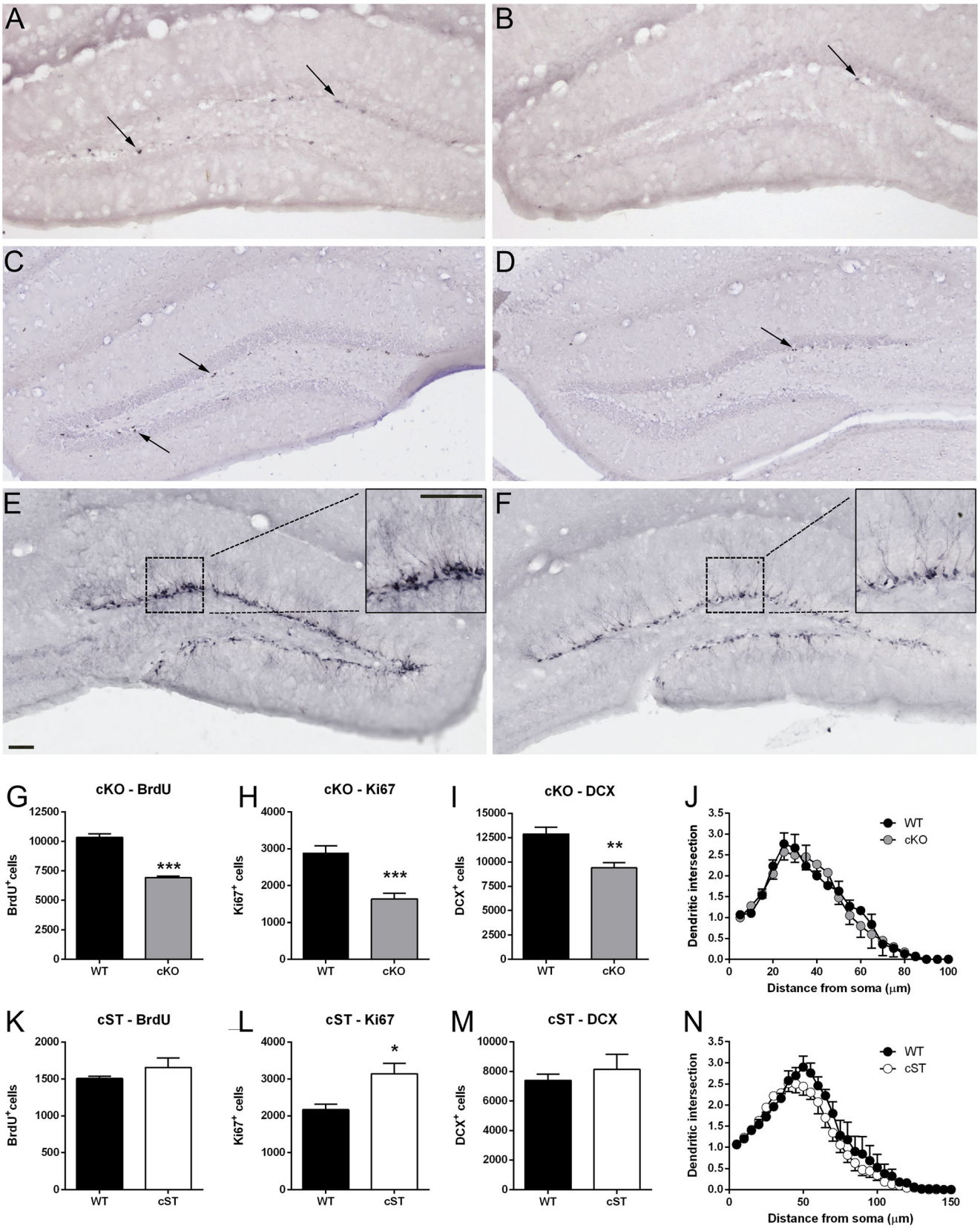


Fig. 4 Assessment of depressive-like behavior in β-catenin inactivated (**a**, **b**) and stabilized (**c**, **d**) mice and their respective WT counterparts. Sucrose preference test in the cKO and cST animals (**a**, **c**). Immobility time in the FST in the cKO and cST mice, measured during the last 4 min in 2-min intervals (**b**, **d**). Data expressed as the mean ± SEM. Student's *t* test; $*p < 0.05$, $**p < 0.01$. $n = 9$–11 animals *per* group



that are positive for GFAP and Sox2 [33] ($p < 0.001$), the number of radial neural stem cells (NSCs) ($p < 0.01$), and horizontal NSCs ($p < 0.01$), compared to their respective WT counterparts (Fig. 7f–h, respectively). The ratio radial/

◀ **Fig. 5** Representative photographs of hippocampal DG in β-catenin inactivated mice: BrdU$^+$ cells in WT and cKO mice (**a**, **b**, respectively), Ki-67$^+$ cells in WT and cKO mice (**c**, **d**, respectively), and DCX$^+$ cells in WT and cKO mice (**e**, **f**, respectively). Scale bar: 100 μm. Graphs showing BrdU$^+$ (**g**, **k**), Ki-67$^+$ (**h**, **l**), DCX$^+$ cells (**i**, **m**), and the dendritic intersection numbers of DCX-positive neurons evaluated by Sholl analysis (**j**, **n**) in β-catenin cKO (**g**–**j**) and cST (**k**–**n**) mice, and their corresponding wild-type counterparts. Data are expressed as the mean ± SEM. The statistical analysis was performed using a Student's *t* test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. $n = 5$–8 animals *per* group

horizontal NSCs did not present differences in the cKO group (Fig. 7i). Mice stabilizing β-catenin also showed an important decrease in the number of cells expressing both Sox2 and GFAP markers ($p < 0.001$), the number of radial NSCs ($p < 0.01$), and the number of horizontal NSCs ($p < 0.001$), compared to their WT counterparts (Fig. 7j–l, respectively). These animals presented an increase in the ratio radial/horizontal NSCs in the β-catenin cST group ($p < 0.01$) (Fig. 7m).

Effect of Chronic Corticosterone Administration in β-Catenin cST Mice

In order to validate the importance of β-catenin stabilization (cST mice) in relation to depressive-like behavior, we evaluated its effect in a mouse model of depression induced by the chronic administration of corticosterone [19] (Fig. 1b).

In the NSF, the cST vehicle mice showed a strong decrease in the latency of feeding compared with WT mice ($p < 0.01$) (Fig. 8a). The chronic administration of corticosterone induced an increase in the latency of feeding in the WT group ($p < 0.05$), and in the cST mice ($p < 0.05$). This latency in the cST animals treated with corticosterone was significantly lower than the latency in WT + CORT group ($p < 0.05$). A two-way ANOVA analysis of the data revealed a significant effect of the corticosterone model [$F(1,31) = 9.21$, $p < 0.01$] and the genotype [$F(1,31) = 17.98$, $p < 0.001$].

In the FST, we observed a lower immobility time in cST animals compared to the WT group ($p < 0.05$) (Fig. 8b). The chronic corticosterone model did not induce changes in immobility in the β-catenin cST mice, compared to the increased immobility observed in the WT animals ($p < 0.05$). Moreover, the immobility time in the WT animals treated with corticosterone was higher than in stabilized β-catenin mice ($p < 0.01$). A two-way ANOVA analysis of the data revealed a significant effect of the corticosterone model [$F(1,32) = 5.80$, $p < 0.05$] and the genotype [$F(1,32) = 20.30$, $p < 0.001$].

The sucrose preference test showed an anhedonic effect in the corticosterone model in the WT animals, compared with their vehicle group ($p < 0.01$) (Fig. 8c). The corticosterone model did not induce anhedonia in the animals with stabilized β-catenin. Moreover, the sucrose preference was higher in the cST + CORT animals compared to the WT + CORT group ($p < 0.05$). A two-way ANOVA analysis of the data revealed a significant effect of the corticosterone model [$F(1,32) = 13.34$, $p < 0.001$].

The analysis of DCX-positive cells in the SGZ of the hippocampus showed a decreased number of DCX-positive cells in the cST compared to their WT counterparts 2 months after the induction of the transgenic animal in the vehicle group ($p < 0.001$) and the CORT animal model ($p < 0.01$). The administration of chronic corticosterone induced a decrease in DCX$^+$ cells ($p < 0.001$) in wild-type animals, while no changes were observed in the cST animals (Fig. 8d). A two-way ANOVA analysis of the data revealed a significant effect of the corticosterone model [$F(1,23) = 13.05$, $p < 0.01$], the genotype [$F(1,23) = 64.32$, $p < 0.001$], and the interaction genotype × corticosterone model [$F(1,23) = 6.94$, $p < 0.05$].

Discussion

The animals used in this study present the inactivation or stabilization of β-catenin in cells expressing the GLAST, which drives efficient recombination in adult neural progenitors [24, 31]. This glutamate transporter is highly expressed in neural

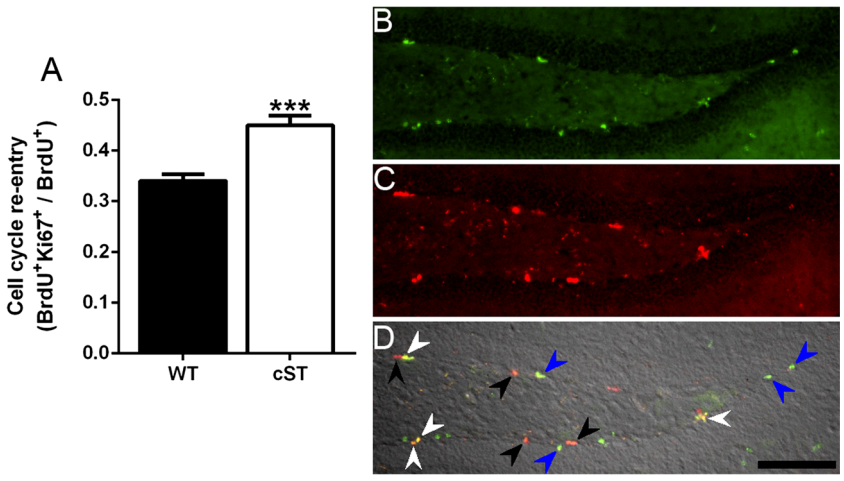


Fig. 6 Cell cycle re-entry (**a**) in β-catenin cST mice 24 h after BrdU administration. Representative images of the BrdU$^+$ cells (**b**), Ki-67$^+$ cells (**c**), and the merge by Nomarsky interference contrast (**d**). In **d**, blue arrows show the BrdU$^+$ cells, black arrows show the Ki-67$^+$ cells, and white arrows show the colocalization BrdU$^+$/Ki-67$^+$ cells. Scale bar: 100 μm. Data are expressed as the mean ± SEM. The statistical analysis was performed using a Student's *t* test; ***$p < 0.001$. $n = 5$–6 animals *per* group

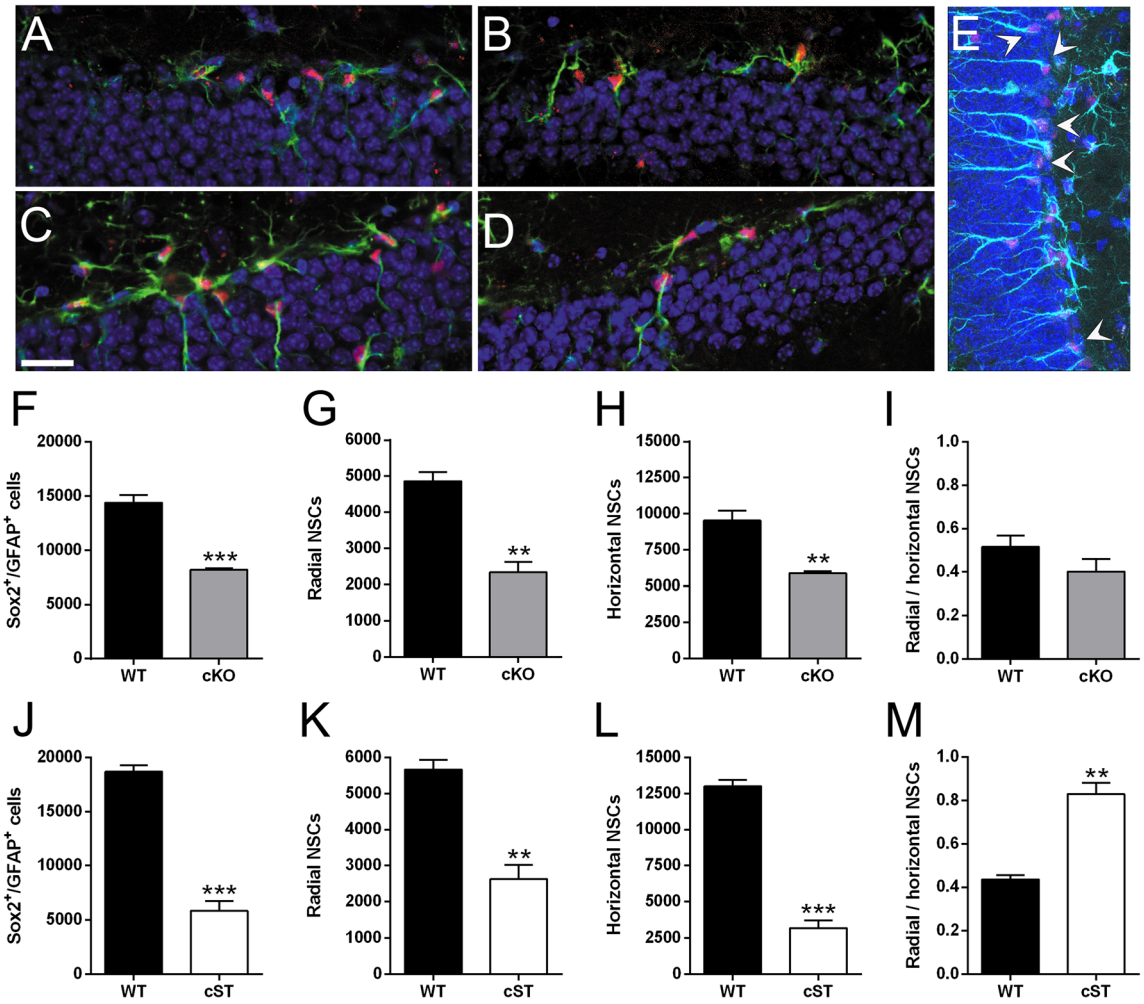


Fig. 7 Representative images of Sox2 (red) and GFAP (green) positive cells in the dentate gyrus of the hippocampus of β-catenin cKO (**b**), and cST (**d**) mice, and their respective WT counterparts (**a**, **c**, respectively). Cell nuclei were stained with DAPI (blue). Cells with radial processes (white arrows) representing radial neural stem cells (NSCs) are indicated in **e**. Scale bar: 20 μm. Graphs showing the number of neural progenitor cells ($Sox2^+/GFAP^+$ cells), in β-catenin cKO (**f**) and cST (**j**) animals, the number of neural stem cells with radial morphology in β-catenin cKO (**g**) and cST (**k**) animals, the number of neural stem cells with horizontal morphology in β-catenin cKO (**h**) and cST (**l**) mice, and the ratio radial/horizontal NSCs in cKO (**i**) and cST (**m**) mice. Data are expressed as mean ± SEM. The statistical analysis was performed using a Student's *t* test; $^{**}p < 0.01$, $^{***}p < 0.001$. $n = 3$ animals per group

stem cells [34], and at a lower expression rate in astrocytes [35]. Although the Cre recombinase in our animals was mainly expressed in the subgranular zone of the dentate gyrus of the hippocampus (Supplementary Fig. S1), a residual expression in astrocytes may be modulating β-catenin levels in other brain areas, which has been linked to a regulatory role of glutamate uptake [36], which could contribute to their behavioral phenotype. The presence of the residual β-catenin-positive cells in the SGZ of the hippocampal dentate gyrus in the β-catenin cKO mice may be due to the incomplete recombination of the floxed sequence induced by the Cre enzyme [37], the existence of differentiated cells expressing β-catenin, or β-catenin localized in cells not expressing GLAST. On the other hand, the animals with the stabilized form of β-catenin in the GLAST-expressing cells present both the wild-type and the deleted (without exon 3) forms of the protein [25], which cannot be differentiated by immunohistochemistry, explaining the lack of changes in the levels of β-catenin protein.

The behavioral and molecular changes in mice after conditional inactivation or stabilization of β-catenin in GLAST-expressing cells were evaluated 1 month after tamoxifen administration. This time point was chosen as 1 month is the time required for neuronal maturation [23], and for classic antidepressants to elicit their therapeutic effect [16].

The inactivation or the stabilization of β-catenin in cells expressing GLAST, mainly localized in the subgranular zone of the dentate gyrus of the hippocampus, has opposite anxiety- and depressive-like behavioral consequences in some of the tests used. The inactivation of β-catenin in these cells resulted in a clear anxious/depressive-like behavior, which correlates with the reduction in β-catenin levels reported in animal models of depression as the chronic unpredictable stress

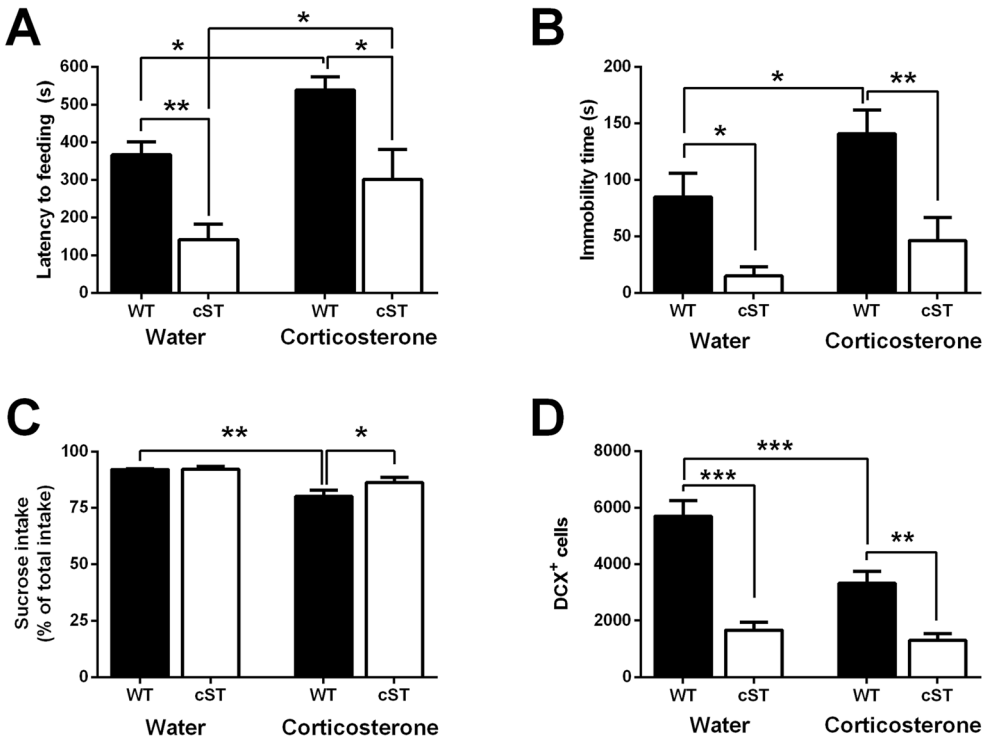


Fig. 8 Anxiety and depressive-like behavior in β-catenin cST mice treated chronically with corticosterone: novelty suppressed feeding test (**a**), forced swimming test (**b**), sucrose intake test (**c**), and neurogenesis evaluated by doublecortin immunolabeling in the SGZ of the hippocampus (**d**). Note that the β-catenin cST animals present a less anxious/depressive behavior than the WT animals in the chronic corticosterone model of depression. Data are expressed as the mean ± SEM. The statistical analysis was performed using a two-way ANOVA, followed by a Newman-Keuls post hoc test; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. $n = 7–10$ animals per group

model [38], pathologies that account for depressive-like behavior [39], and postmortem prefrontal cortex and nucleus accumbens samples from major depressive patients [6, 7, 40]. Previous studies using β-catenin knockout animals in forebrain, or the downregulation of the pathway [41, 42], have also reported a depressive-like behavioral phenotype in the tail suspension test [43]. Moreover, we also demonstrate an anxiogenic effect due to the inactivation of β-catenin in GLAST-expressing cells, mainly localized in the subgranular zone of the dentate gyrus of the hippocampus, although no changes in anxiety have been reported after β-catenin elimination in forebrain [43].

Conversely, the animals with stabilized β-catenin not only presented an anxiolytic-like response in the novelty suppressed feeding test but also are less prone to show depression-like behaviors (i.e., in forced swimming test) when subjected to a depression model as the chronic corticosterone administration paradigm [27]. Specifically, the novelty suppressed feeding test is a neurogenesis-dependent and a predictive behavioral paradigm of both anxiolytic and antidepressant-like effects [16, 17, 44], paralleling the clinical outcome in humans following chronic treatments with antidepressants [45, 46]. Transgenic mice overexpressing a constitutively active form of β-catenin in the mouse brain [47], or with upregulated Wnt/β-catenin pathway [41, 48], present an antidepressant-like phenotype evaluated in the FST. Moreover, the administration of classic antidepressants or drugs with antidepressant effect—as 5-HT_4 partial agonists—is also associated with an increase in β-catenin localized in progenitor cell niches in the SGZ of the hippocampus [10, 11]. All these behavioral manifestations in stabilized β-catenin mice may account for their stress resilience phenotype. Indeed, the stabilization of β-catenin in the SGZ of the dentate gyrus of the hippocampus conferred a "protective" feature against the anxious/depressive-like behavior characteristic of the chronic corticosterone model of depression, in different behavioral paradigms. This pro-resilience effect has been associated with the upregulation of β-catenin signaling in the hippocampus [41], and in the nucleus accumbens [40], and more generally, with treatments that promote adult neurogenesis [49, 50]. This resilient phenotype in cST animals can be associated to the lack of downregulation in doublecortin labeling after corticosterone administration, as reported in animals with increased hippocampal neurogenesis [51]. It is puzzling the convergence of an anti-depressive-like behavior in cST mice, parallel to a reduction in doublecortin in cST animals 2 months after the transgenic induction. So, other mechanisms as the neurotransmitter trafficking may be playing a role in this behavioral outcome since the Wnt signaling plays an important modulatory role in this process [52].

The inactivation of β-catenin in GLAST-positive cells of the SGZ of the hippocampus resulted in deep changes of SGZ populations from the different stages analyzed in this study. The decrease in proliferation markers, immature neurons, and neural progenitors has been previously described in animals lacking β-catenin [53]. This reduction is proportional to the number of astrocytes that express lacZ in the subgranular zone of the dentate gyrus in our animals. Furthermore, the reduction

in β-catenin is associated with defects in dendritic morphology in post-natal born dentate gyrus granular neurons [54]. While the lack of changes in the number of intersections, indicative of dendritic complexity in newborn granule cells, in our β-catenin cKO mice, are in agreement with animal models showing impaired hippocampal proliferation [15]. These effects on proliferation support the importance of β-catenin in its control [55, 56].

By contrast, the animals with a stabilized form of β-catenin showed results with a more complex interpretation. The proliferation marker Ki-67 was significantly increased with no changes in BrdU or DCX immunostaining. In progenitor cells, after an initial increase of β-catenin to promote proliferation [21, 53], a later decrease is needed to initiate differentiation [57]. The immature cells in our cST animals may come predominantly from the non-recombined progenitor pool, leading to a lack of changes in dendritic complexity. The apparent discrepancy between proliferation markers' labeling (BrdU and Ki-67) reflects that 1 month after transgenic induction, the number of cells that enter in the cell division phase (BrdU labeling) remains constant compared with the wild-type group, while a general increase in the number of proliferating cells can be found after Ki-67 staining. This was confirmed by the increase observed in the cell cycle re-entry in the animals that stabilize β-catenin. In this sense, different authors have reported that the overexpression of β-catenin inhibits the cellular differentiation, reducing the capacity of the progenitor cells to exit the cell cycle [21, 58].

The reduction in neural progenitor cells observed after β-catenin elimination in GLAST^{+} cells is associated to a similar decrease of radial and horizontal neural stem cells. In contrast, in the animal that stabilizes β-catenin, the reduction in the number of horizontal stem cells is higher than for radial glial progenitor cells, suggesting that the rate of disappearance is different for the radial glial cells and for the horizontal neural progenitors. The increased ratio radial/horizontal NSCs may be associated to the maintenance of the pool of radial glial cells through symmetric division, as reported following the activation of Wnt signaling pathway [59]. Furthermore, the reduction in the number of radial stem cells has been associated with a decrease in the progenitor pool after a series of asymmetrical divisions, as observed during the aging process, which may lead to an increase in astrocytes as a result of the progenitor pool exhaustion [60]. However, we cannot discard that according to studies in vivo [53, 57], and in vitro [61], the stabilization of β-catenin leads to increased apoptosis shortly after the overexpression.

The moderate hippocampal β-catenin increase observed only shortly after the transgenic induction, together with the low raise in proliferative neurogenic markers after β-catenin stabilization, suggests a tight control of these processes. Since our mouse model is not an isolated system, it is expected that 1 month after transient manipulation of β-catenin in GLAST-expressing localized in the neural stem cells of the SGZ [34], the Wnt/β-catenin pathway would be subjected to the regulatory machinery present in the neurogenic niche. In this sense, Wnts produced within the progenitor niche are involved in neurogenesis and maturation of hippocampal neurons [55, 56, 62].

The behavior of inactivated β-catenin mice parallels their impaired hippocampal proliferation, whereas this is not the case in stabilized β-catenin animals in which the cellular changes are not so apparent, suggesting the involvement of additional mechanisms, for instance, increased synaptic plasticity by means of β-catenin/N-cadherin complexes [63, 64]. In addition, N-cadherin/β-catenin complexes regulate proliferation and differentiation of neural stem cells in the developing neocortex [65], which may also contribute to the control of proliferation in adult neural progenitors.

In sum, β-catenin signaling in areas as the SGZ of the hippocampus plays a necessary role to modulate fundamental aspects of the proliferation in the adult brain that appears to be contributing to the anxious- and depressive-like behavior. Moreover, β-catenin stabilization in GLAST-expressing cells that are densely present in the SGZ of the hippocampus is associated with resilience in animal models of depression, supporting its implication in the pathophysiology of depression. However, additional studies are necessary in order to gather evidences of the interactions of the β-catenin signaling pathway with other components of its canonical pathway or with other extrinsic factors such as bone morphogenetic protein (BMP), Notch, and Sonic hedgehog (Shh) that participate in both neurogenesis and behavioral responses in order to unravel the road up to the therapeutic target.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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