CRITICAL ROLE OF ADULT-BORN DENTATE GRANULE NEURONS IN PATTERN COMPLETION'

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Abstract:

The dentate gyrus contains neurons generated during development and adulthood, yet their distinct roles remain unclear. Using a transgenic mouse model, we show that adult-born neurons contribute to pattern completion, essential for episodic memory. This has implications for aging, PTSD, and addiction, where excessive pattern completion leads to maladaptive memories. Targeting ABNs could offer new therapeutic strategies to regulate memory processes and improve mental health.

The recollection of memories relies on several interconnected cognitive processes. After encoding—during which new information is integrated with existing knowledge—memories are stabilized and stored over time. Accessing and reconstructing past experiences depends on various mechanisms, including pattern completion. Pattern completion allows the brain to retrieve a complete memory from partial or incomplete cues [1,2].

The dentate gyrus, particularly neurons generated during adulthood, has been implicated in memory recollection. Research has shown that ablating, silencing or inhibiting these neurons impairs memory retrieval [3-5], while stimulating their activity during retrieval enhances the accuracy of long-term memory recall [6]. Although these findings suggest that adult-born neurons are both necessary and sufficient for accessing intact stored memories., their role in reconstructing a complete memory from partial cues has not been yet investigated.

To this aim, we used an already established inducible transgenic mouse model to stop the production of new neurons[7,8]. In this model, Ascl1, a bHLH transcription factor involved in cell cycle exit and proliferation, is deleted in radial glial cells by crossing mice carrying the transgenes *Glast-CreERT2* and *Ascl1* $^{fl/fl}$ (FF) and administering tamoxifen (Tam) to the double transgenic mice. Adult neurogenesis was examined in WT, *Glast-CreERT2*, *Ascl1* $^{fl/fl}$ (FF) and *Glast-CreERT2 x Ascl1* $^{fl/fl}$ (cKO) mice treated with Tam at 2 months of age. CldU was injected 1 month later to verify the efficacy of Tam treatment at this early time point. Three months after CldU injection, we analyzed the number of CldU label-retaining cells and the number of immature neurons expressing doublecortin. Because cell numbers in the WT and Glast-CreERT2 -Tam groups were similar (Figure S1), they were pooled as a single control group (Ctl-Tam group).

As expected, the number of CldU-label-retaining cells was dramatically decreased in cKO-Tam mice compared to the other groups. This confirms that one month after Tam injections, neurogenesis was abolished in cKO-Tam mice (Figure 1a $F_{2,27}$=20.61, p<0.0001). In FF-Tam mice, which do not carry the *Glast-CreERT2* construct, the number of CldU-IR cells was lower than in Ctl-Tam group (p=0.018), although it did not significantly differ from the cKO-Tam mice (p=0.27). This phenomenon previously observed[7] suggested that the *Ascl1* $^{fl/fl}$ allele is hypomorphic and expresses *Ascl1* at a level lower than WT, leading to reduced neurogenesis in *Ascl1* $^{fl/fl}$ animals even in the absence of recombination.

Next, the impact of removal of *Ascl1* in dentate gyrus stem cells was examined using doublecortin (Dcx) as a surrogate of immature neurons. In line with previous results the number

of Dcx-IR cells was significantly lower in FF-Tam mice compared to Ctl-Tam mice (Figure 1b $F_{2,27}$=20.57, p<0.0001, Ctl-Tam vs FF-Tam, p=0.03), although it remained higher than in cKO-Tam mice (FF-Tam vs cKo-Tam, p=0.02).

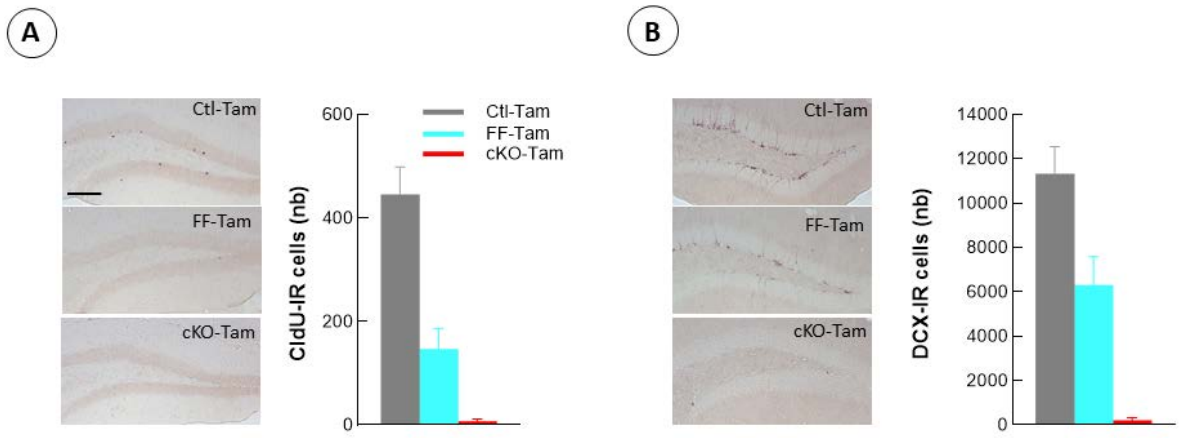

Figure 1. Removal of *Ascl1* in stem cells reduces adult neurogenesis. (A) The total number of CldU-IR cells and (B) the total number of Dcx-IR cells was quantified in the granule cell layer (GCL) of the dentate gyrus. Data are mean ± SEM. Scale bar = 100 µm.

Pattern completion was measured two months after Tam injection in the Morris water maze as previously described[8] (Figure 2A). After a familiarization phase, mice were trained to find a hidden platform from different departure points. During this phase (sessions 1 to 4, Figure S2), all groups of mice learned to find the hidden platform [$F_{3,102}$=12.713; p<0.01].

Then we investigated the role of the adult-born neurons in long-term retrieval in pattern completion condition where three of the principal cues were removed and a probe test was conducted. We first analyzed the crossing of different annuli. As a significant group x quadrant interaction was found (see figure 2B), a complementary analysis was conducted within each group of mice. A significant quadrant effect was observed in the Ctl-Tam group [$F_{3,72}$=14.391, p<0.001] and post-hoc analyses revealed that the target annulus was crossed more frequently than the other three (p<0.001). In the FF-Tam group, a quadrant effect was also found [$F_{3,36}$=3.252, p=0.033], but post-hoc analysis revealed a significant difference only between the Target and Adjacent2 quadrants. Finally, in the cKO-Tam group, no quadrant effect was detected [$F_{3,28}$=0.188, p=0.903], with all four annuli being crossed at similar frequencies.

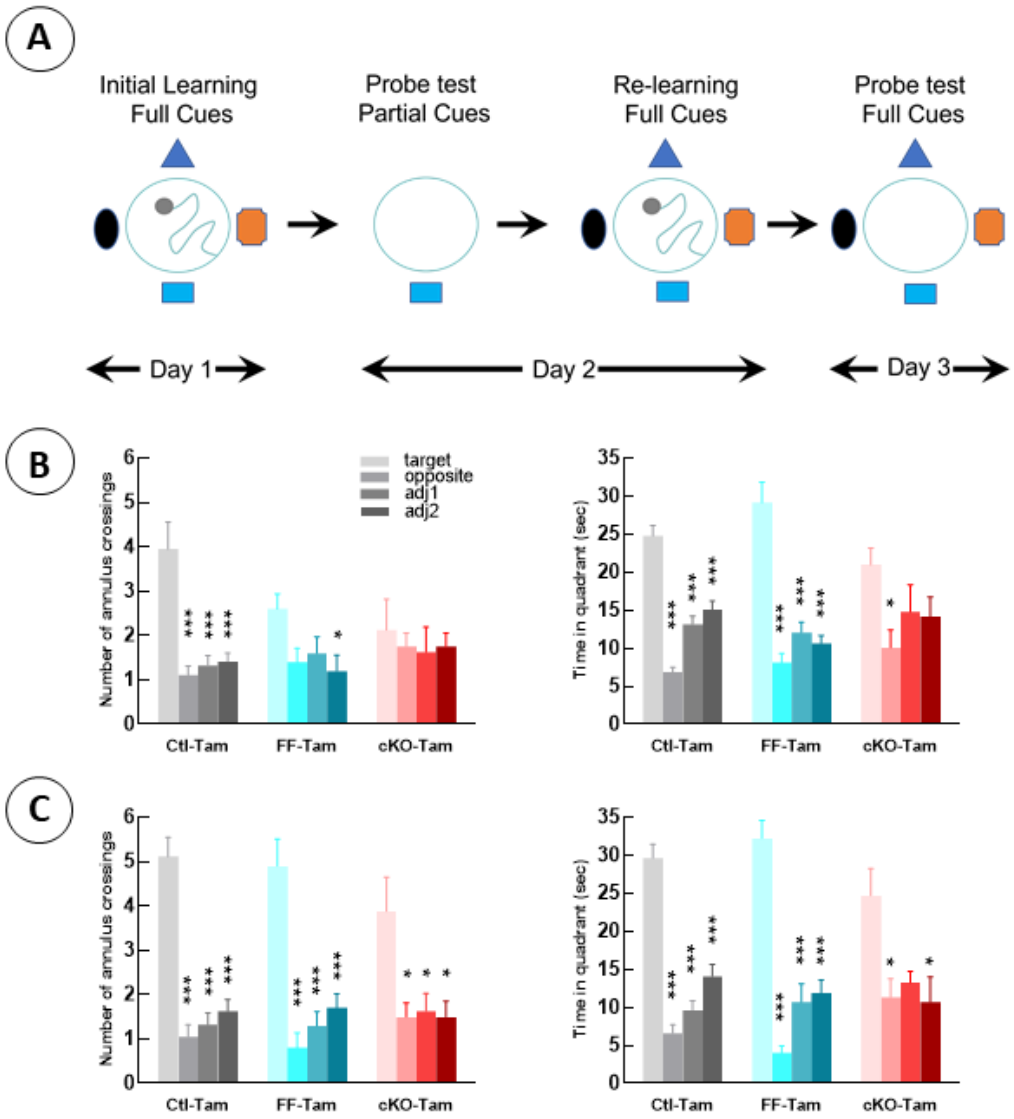

Figure 2. Removal of *Ascl1* in stem cell impairs pattern completion. (A) Experimental protocol. (B) Pattern completion measured during a probe test with partial spatial cues. We observed a significant quadrant effect for the number of annulus crossings, [$F_{3,136}$=8.358; p<0.001] and for time in the Target quadrant [$F_{3,136}$=51.027, p<0.001] no main effect of group for both behavioral measures [$F_{2,136}$ =1.03 p=0.420; $F_{2,136}$ =0.001 p=0.999] but a significant group x quadrant interaction for the number of annulus crossings [$F_{6,136}$=2.242; p=0.043] and for the time in quadrants [$F_{6,136}$=2.776; p=0.014].Thus, mice from the three groups did not explore the four quadrants in the same way. (C) Spatial probe test when all cues were reintroduced. *** p<0.001, ** p<0.01, * p<0.05 Target vs other.

For the time in quadrants, we also performed a complementary analysis within each group. Indeed, in the Ctl- Tam group and the FF- Tam group, a significant quadrant effect was

observed [$F_{3,72}$=47.249, p<0.001; $F_{3,36}$=31.622, p<0.001 respectively]. Post-hoc analyses for both groups revealed that the mice spent significantly more time in the Target quadrant than in the other three (p<0.001). In contrast, in the cKO- Tam group, the quadrant effect was not significant [$F_{3,28}$=2.729, p=0.063], with a significant difference observed only between the Target and Opposite quadrants.

Thus, when the environment was significantly impoverished, the control group was still able to locate the platform in the correct location. This was evident both in the annulus crossings and the time spent in each quadrant. The FF-Tam group was also able to perform pattern completion, but their performance was reduced compared to the control group: they could still search for the platform in the correct quadrant (time in quadrant), but they were no longer able to precisely locate the platform (annulus crossings). In contrast, the cKO-Tam group appeared disoriented in the pattern completion condition and no differences were observed between the number of crossings of the four annuli. Platform search behavior seemed random, although a significant difference in time spent in the Target and Opposite quadrants was still observed.

Then in a final step, we asked wether memory retrieval was preserved when all cues were present. To do so, mice were first re-trained with all spatial cues (Figure S2), followed by a probe test with all the cues. During the relearning phase (sessions 5 to 6) analyses showed no significant effect of group [$F_{2,34}$=0.293; p =0.748] or interaction between group and relearning sessions [$F_{2,34}$=0.411; p =0.667].

During the probe test with all cues, for the annulus crossing there was no main effect of group [$F_{2,136}$=0.156, p=0.855] and no group x quadrant interaction [$F_{6,136}$=0.951, p=0.461]. For the time by quadrant, there was no main effect of group [$F_{2,136}$=0.022, p=0.855] but a group x quadrant interaction [$F_{6,136}$=2.955, p=0.010]. For all three groups of mice, post-hoc analyses revealed that the mice crossed more frequently the target annulus and spent significantly more time in the Target quadrant compared to the other three quadrants (Figure 2C) indicating a good spatial memory.

In the same mice we verified that deficits in pattern completion were not related to motor or motivation deficits by analysis swim speed that was similar between groups during the probe tests [$F_{2,34}$=0.275; p =0.761]. These data confirm that ablating adult-born neurons impairs the capacity to pattern completion recall. Finally, in a separate experiment, we verified that memory deficits were not related to an alteration of emotional status (Figure S3)

Discussion:

Using an established transgenic mice model to block the production of adult born neurons, we highlighted for the first time that ablating adult-born neurons led to a specific alteration of pattern completion. Indeed, both using the number of annuli crossing and the time spend in the target quadrant, cKO-Tam group were impaired in reconstructing a complete memory from partial cues. These deficits were not due to the speed of pattern completion recall or to anxiety-like or depression-like behavior. Interestingly, a partial reduction of neurogenesis in FF-Tam mice was insufficient to disrupt pattern completion, although performances were not as good as controls (annuli crossing).

Previous studies have shown that the CA3 region onto which dentate granule neurons project through their mossy fibers (MF) plays an important role in spatial pattern completion. More specifically, a crucial role of NMDA receptors in CA3 was evidenced using lesion, genetic and pharmacological approaches [9-11]. Previous studies have shown that mature dentate granule neurons play an important role as their genetic ablation impaired the speed of pattern completion recall (and not its capacity [12]). But whether the temporal origin of these matures neurons—developmental or adult-born—affects this role has remained unclear. Our results show for the first time that adult-born neurons are involved in pattern completion. This role together with their ability to sustain pattern separation [12-14] and relational processing [15] allow to propose that adult-born neurons play a key role in episodic memory by storing distinct experiences, retrieving them efficiently, and connecting them meaningfully.

Our findings have significant implications for aging, during which pattern completion declines. Additionally, maladaptive memories—driven by excessive pattern completion—are central to post-traumatic stress disorder (PTSD) and addiction. In PTSD, minor, non-threatening cues can trigger vivid re-experiencing of traumatic memories, while in addiction, exposure to drug-related cues can induce powerful cravings by reactivating drug-use memories. Given the role of adult-born neurons in these conditions, our discovery highlights adult-born neurons as potential therapeutic targets to modulate pattern completion, offering new avenues for improving mental health.

Resource availability

All data are available in the main text or supplementary materials or upon request to the corresponding author.

Acknowledgments

We thank the animal facility and the genotyping platform of INSERM U1215 NeuroCentre Magendie, funded by INSERM and Labex Brain, for animal care and mouse genotyping. More specifically the help of Cédric Dupuy, Fiona Corailler, and Delphine Gonzales is acknowledged. This work was supported by Institut National de la Santé et de la Recherche Médicale, INSERM (to DNA), Centre National de la Recherche Scientifique, CNRS (to MK), and the Francis Crick Institute, which receives its funding from Cancer Research UK (FC CC2033), the UK Medical Research Council (FC CC2033), and the Wellcome Trust (FC CC2033).

Author contributions

PR, MK and DNA conceived experiments. PR and PD performed experiments. PR and MK analyzed data. PR, MK and DNA wrote original draft. FG provided biological resources. All authors contributed to the article and approved the submitted version.

Declaration of interest

The authors declare no conflict of interest.

MATERIAL AND METHODS

A . Animals

Adult male double transgenic mice containing a conditional mutant allele of *Ascl1* and the *Glast-CreERT2* allele were used in the experiments. Ascl1 is a proneural transcription factor, expressed by adult stem cells, promoting their activation, proliferation and differentiation into neurons via the generation of intermediate progenitors [7]. The conditional mutant allele of *Ascl1* (*Ascl1* $^{fl/fl}$) consists of the coding sequence of *Ascl1* flanked by loxP sites on both ends allowing its deletion by the Cre recombinase. The Cre recombinase is fused with modified estrogen receptor (ERT2) that restricts the enzyme in the cytoplasm, activating its translocation to the nucleus to mediate recombination only upon Tam stimulation [16]. Cre-ERT2 is expressed under the GLAST promoter, which is a marker for adult neural stem cells. The double transgenic mice containing both the alleles was obtained from the breeding of heterozygous male mice carrying the Glast-CreERT2 construct (Glast-CreERT2 mice) with female mice homozygous for the floxed *Ascl1* construct (*Ascl1* $^{fl/fl}$ mice = FF mice), as previously described [7,16]. All experimental procedures were carried out following the European directives of 24 November 1986 (86/609/ECC) that was in effect at the time of the experiments. Animal studies were approved by the ethical committee of Bordeaux (CEEA50; Dir 1399, A16502).

Mouse lines used in this study:

WT	*Glast-CreERT2 -/- ; Ascl1* $^{+/+}$ (+Tam)
Glast-CreERT2	*Glast-CreERT2 +/-; Ascl1* $^{+/+}$ (+Tam)
FF group	*Glast-CreERT2 -/- ; Ascl1* $^{fl/fl}$ (+Tam)
cKO group	*Glast-CreERT2 +/- ; Ascl1* $^{fl/fl}$ (+Tam)

B. Tamoxifen treatment

Two-month-old mice were administered intraperitoneally (i.p.) with tamoxifen (Tam) for 5 consecutive days at a concentration of 100mg/kg body weight.

C. Injection of CldU

One month after Tam injection, mice were injected with Choro-2'-Desoxyuridine (CldU, 42.75 mg/kg dissolved in 0.9% NaCl), once per day during 2 days. Animals were sacrificed three months after Tam injection.

D. Immunohistochemistry and stereology

Animals were anesthetized and perfused transcardially with 0.1 M phosphate buffered saline (PBS, pH 7.4), followed by 4% buffered paraformaldehyde (PFA). Brains were collected and post-fixed in PFA at 4 °C for a week. Subsequently, 40 µm-thick coronal sections were cut using a vibratome (Leica) and stored in cryoprotectant medium (30% ethylene glycol, 30% glycerol in KPBS) at −20 °C before staining.

Free-floating sections were processed in a standard immunohistochemical procedure in order to visualize CldU (Anti-BrdU, 1/1000, Accurate OBT0030) or doublecortin (Dcx, 1:8000; Sigma D9818)-labeled cells. Briefly, sections for CldU staining were exposed to HCl (2N) for 30 min at 37°C and rinsed with PBS, while for Dcx, sections were directly rinsed in PBS. Then all sections were treated with methanol and 0.5% H2O2 for 30 min. Sections were washed again in PBS before incubation with a blocking solution containing 3% normal serum and 0.3% Triton X100 in PBS for 45 min at room temperature. They were then incubated for 48 h at 4 °C with the primary antibodies diluted in the blocking buffer. Then sections were incubated with biotin-labeled secondary antibodies diluted in PBS—0.3% Triton X100—1% normal serum, and immunoreactivities were visualized by the biotin–streptavidin-peroxydase technique (ABC Hrp kit; Dako) with 3,3′-diaminobenzidine (DAB) as chromogen. The number of immunoreactive (IR) cells throughout the entire granule and subgranular layers of the left DG was estimated using the optical fractionator method as previously described[17].

E. Pattern completion

1 . Animals

In this experiment, 37 mice were treated with Tam at 2-months of age. Three experimental groups were constituted: a Control Tam group (n=19, with 11 WT and 8 *Glast-CreERT2*), a Flox-Tam group (*Ascl1* $^{fl/fl}$-TAM; n=10) and a cKO-TAM (8 *Glast-CreERT2* x *Ascl1* $^{fl/fl}$ Tam). Two months later, these mice were trained in the water maze.

2 . Task apparatus

The water maze was an ivory circular pool (110 cm diameter, 30 cm high) filled to a depth of 15 cm with water maintained at 23 ± 1°C. The water was made opaque by addition of a white opacifier. A white-painted platform (9 cm diameter) was placed inside the pool, 15.5 cm away from the pool wall. The apparatus was surrounded by white curtains preventing mice from using visual extra-maze cues and was illuminated by three lamps heterogeneously placed behind the curtains[18]. Four white and black visual landmarks approximately located at 50 to 100 cm

from the pool, differing in shapes, motives and size, were placed on the curtains (Fig. 1A). The swimming pool was surmounted by a video camera connected to a video recorder and a computerized tracking system (Ethovision®, Noldus).

3. Behavioral Procedures

On day 0, mice were individually submitted to a single familiarization session of three trials with the platform located always in the centre of the pool and protruding 0.5 cm over the surface of the water. The starting positions were determined in a pseudorandom order, such that each of them was used once in a single session. If the mouse did not find the platform within one minute, it was gently guided to it. The animal stayed one minute on the platform between the trials and before returning to the home cage. All mice showed similar latencies in finding the visible platform [data not shown, $F(2,34) = 0.232$; $p = 0.794$].

On day 1, mice were given four consecutive acquisition sessions of three trials with an inter-session delay of 25-30 min during which they were returned to their home cage. The procedure was the same as in the familiarization phase, except that the platform was placed in a specific quadrant (target quadrant) and was submerged 0.5 cm beneath the surface of the water.

On day 2, the platform was removed from the pool and mice were submitted to a 60-sec probe-trial. During this first probe trial (PT1), the three largest patterns were removed, thus only the smallest one was maintained. Thirty min after this probe trial, mice received 2 sessions of reacquisition in the standard condition with the platform in the target quadrant and with all patterns around the pool.

On day 3, mice were submitted a second 60-sec probe-trial (PT2), but this time, all cues were present.

F . Emotional behaviors

1 . Animals

In this experiment, 48 mice were treated with Tam at 2-months of age. Three groups of mice treated with Tam were used: a Ctl group (n=23, with 13 WT and 10 Glast-CreERT2), a FF group (n =11) and a cKO group14 (n=14). Behavioral analysis began 2 months after Tam injection. Mice were placed into individual cages one week before the first test session.

2 . Anxiety-related behaviors

The impact of removing *Ascl1* in RGLs was first examined on anxiety-related behavior by measuring avoidance responses to potentially threatening situations, such as unfamiliar open environments[17]. Mice were first tested in an *open-field* (OF; 50 x 50 cm white PVC square arena; ~400 lux) to evaluate locomotor performance and exploratory activity. Mice were placed in the center of the OF and allowed to explore the test for 10 min. Total distance traveled and time spent in the corners (the safest place of the open-field) were recorded and analyzed using a videotracking system (© Videotrack, ViewPoint).

Two days later, a *light/dark emergence test* was conducted in the same open-field (~400 lux) containing a cylinder (10 cm deep, 6.5 cm diameter in dark gray PVC) located length-wise along one wall, its opening facing a corner of the OF. Mice were placed into the cylinder and tested for 15 min. Total time spent inside the cylinder and total number of entries in the cylinder (safe place) were analyzed.

Finally, an *elevated plus maze* (EPM) test was performed 48h later. The test was a transparent Plexiglass apparatus with 2 open (45 x 5 cm) and 2 closed (45 x 5 x 17 cm) arms extending from a common central platform (5 x 5 cm). Mice were released from the central zone, facing an open arm and allowed to freely explore the maze for 5 min (~50 lux). Time spent in and total number of entries into the open and closed arms were measured. Standard measures of rodent anxiety were calculated: % time and % entry in the open arms compared to time in open arms + closed arms, and total entries into any arm of the maze; in addition, total distance traveled in the open and closed arms was taken as a measure of activity/exploratory tendency.

3 . Depression-related behaviors

Depression-related behaviors were measured in the same animals upon completion of the anxiety tests as previously described [17]. Briefly depression-related behaviors were examined by measuring avolition (lack of motivation or inability to initiate goal-directed behavior) in the nest building and sucrose splash tests, anhedonia in the sucrose preference test, and resignation/behavioral despair in the Forced swim test (FST). At least 4 days separated each test.

Nest building was measured by placing a cotton nestlet in each cage in the morning and scoring nest quality 24 h later.

The ***sucrose splash test*** was carried out by spraying a high viscosity 10% sucrose solution on the coat of the mice to induce a self-grooming behavior. Latency to initiate the first grooming

episode, as well as frequency of grooming over a 5-min period was measured immediately after applying the solution.

For the ***sucrose preference*** test, mice were first habituated for 48 h to the presence of two drinking bottles filled with tap water. They were then given, for 48 h, a free choice between one bottle filled with a 4% sucrose solution, and the other with tap water. Sucrose intake was calculated as the amount of consumed sucrose in mg per gram body weight, and sucrose preference was calculated according to the formula: sucrose preference = (sucrose intake)/(sucrose intake + water intake) × 100.

The ***Forced swim test (FST)*** was performed last in a 5L glass cylinder filled with 26±1°C water to a depth of 20 cm so that mice cannot touch the bottom of the cylinder. One 6-min session was run and both latency to float and duration of immobility over the last 4 min of the test were scored by an experimenter unaware of the experimental groups.

G. Statistical analysis

Statistical analyses were performed with GraphPad Prism 10 software (GraphPad v10.0.3; GraphPad Software LLC). No statistical methods were used to predetermine sample sizes. Investigators were blind to group allocation in behavioral experiments. Graphs represent mean values ± SEM. A 95% confidence interval was used for statistical comparisons.

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Supplementary figures

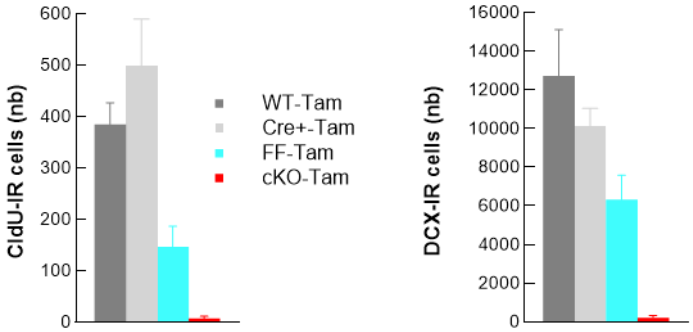

Figure S1: Removal of *Ascl1* in stem cells decreases neurogenesis.
(A) The number of CldU-IR cells varied among the four groups [$F_{3,27}$=14.98, p<0.0001] with WT-Tam and Cre+-Tam showing no significant difference (p=0.498).
(**B**) The number of Dcx-IR cells also varied among the four groups [$F_{3,27}$=14.71, p<0.0001] with WT-Tam and Cre+-Tam showing no significant difference (p = 0.557).

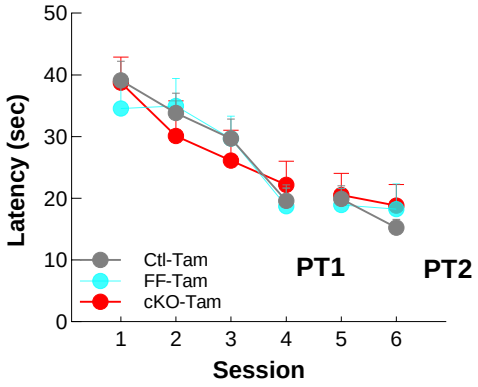

Figure S2: Initial learning and re-learning with all spatial cues.
During the initial learning (S1-S4), all groups learn to find the hidden platform [group effect, $F(3,102) = 12.713$; $p < 0.01$; group x session : $F(6,102) = 0.411$; $p = 0.870$].
During the re-learning phase (sessions 5 to 6), mice were highly efficient. Again, analyses showed no significant effect of group [$F(2,34) = 0.293$; $p = 0.748$] or interaction between groups and reacquisition sessions [$F(2,34) = 0.411$; $p = 0.667$]. PT1: first probe trial with partial cues. PT2: second probe trial with partial full cues.

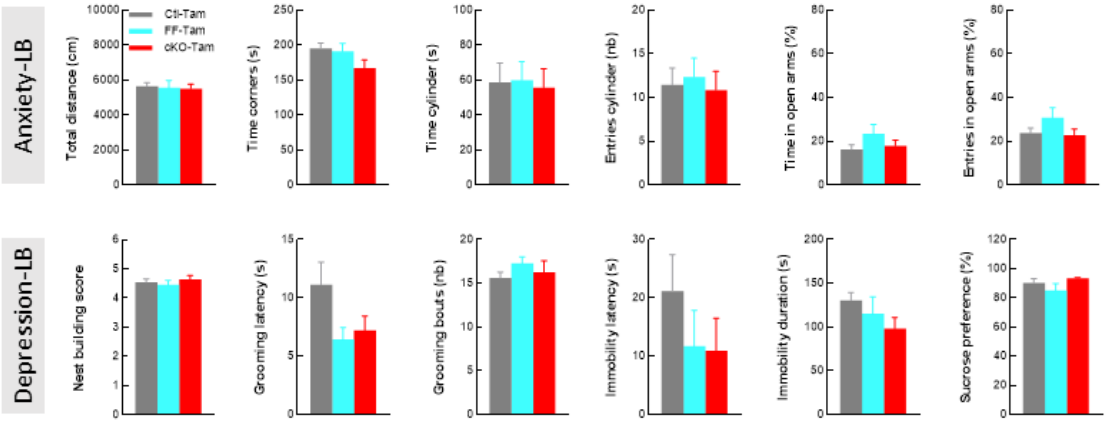

Figure S3. Removal of *Ascl1* in stem cells does not influence anxiety and depression-like behavioral responses.

Anxiety-like responses were measured in the open-field (**a-b**), in the light/dark emergence task (c, g) and in the elevated plus-maze (e,f). Depression-like responses included measures of *motivation* evaluated in the nest building (g) and the sucrose splash tests (h, i), of *resignation* evaluated in the forced swim test (j,k), and of *anhedonia* measured in the sucrose preference test (l)..

Avoidance of threatening areas was similar among groups as shown by the total distance traveled in a bright open-field (**a**; group effect $F_{2,45}$=0.067, p=ns) and the time spent in its safest areas, the corners (**b**; group effect $F_{2,45}$=2.339, p=ns). This was confirmed in a light/dark emergence task, a free exploration task in which animals can explore a brightly illuminated OF or retreat into a dark and reassuring cylinder (**c**; *Time in the cylinder* group effect $F_{2,45}$=0.029, p=ns; **d**; *Entries in the cylinder* group effect $F_{2,45}$=0.097, p=ns), and in the elevated plus maze where the percentage of time spent (**e**; group effect $F_{2,45}$=1.687, p=ns) and the number of visits (**f**; group effect $F_{2,45}$=1.656, p=ns) were similar among the different groups of mice.

For depression-related behavior, no difference between groups could be observed using readouts for lack of motivation such as quality of nest in a nest building task (**g**; group effect $F_{2,45}$=0.386, p=ns), or latency (**h,** group effect $F_{2,45}$=2.177 p=ns) and frequency (**I,** group effect $F_{2,45}$=0.787, p=ns) to groom in a splash test. Resignation in the forced swim test (**j**; *Immobility latency* group effect $F_{2,45}$=0.890, p=ns, **k**; *Immobility duration* group effect $F_{2,45}$=1.837, p=ns) and anhedonia in the sucrose preference test yielded same conclusions (**f**; group effect $F_{2,45}$=1.656, p=ns).