ARTICLE

Hippocampal cytogenesis abrogation impairs inter-regional communication between the hippocampus and prefrontal cortex and promotes the time-dependent manifestation of emotional and cognitive deficits

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Impaired ability to generate new cells in the adult brain has been linked to deficits in multiple emotional and cognitive behavioral domains. However, the mechanisms by which abrogation of adult neural stem cells (NSCs) impacts on brain function remains controversial. We used a transgenic rat line, the GFAP-Tk, to selectively eliminate NSCs and assess repercussions on different behavioral domains. To assess the functional importance of newborn cells in specific developmental stages, two parallel experimental timeframes were adopted: a short- and a long-term timeline, 1 and 4 weeks after the abrogation protocol, respectively. We conducted in vivo electrophysiology to assess the effects of cytogenesis abrogation on the functional properties of the hippocampus and prefrontal cortex, and on their intercommunication. Adult brain cytogenesis abrogation promoted a time-specific installation of behavioral deficits. While the lack of newborn immature hippocampal neuronal and glial cells elicited a behavioral phenotype restricted to hyperanxiety and cognitive rigidity, specific abrogation of mature new neuronal and glial cells promoted the long-term manifestation of a more complex behavioral profile encompassing alterations in anxiety and hedonic behaviors, along with deficits in multiple cognitive modalities. More so, abrogation of 4 to 7-week-old cells resulted in impaired electrophysiological synchrony of neural theta oscillations between the dorsal hippocampus and the medial prefrontal cortex, which are likely to contribute to the described long-term cognitive alterations. Hence, this work provides insight on how newborn neurons and astrocytes display different functional roles throughout different maturation stages, and establishes common ground to reconcile contrasting results that have marked this field.

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INTRODUCTION

Among different forms of plasticity that allow the mammalian central nervous system (CNS) to adapt both structurally and functionally to every day contingencies, its ability to generate new cells from resident neural stem cells (NSCs) has been studied extensively and lies in the center of a still ongoing heated discussion. Despite still being a controversial research topic, proliferation and differentiation of new cells has been shown to occur throughout life in the hippocampus of mammals, including in humans [1–9]. New neurons in the hippocampal dentate gyrus (DG) exhibit distinct properties from their mature counterparts, presenting enhanced synaptic plasticity during a critical period of maturation, 2 and 6 weeks post neuronal birth [10–13]. Astrocytes are also generated from NSCs in vivo, though very little is known regarding their functional properties [14, 15].

This ability to generate new brain cells throughout adulthood, a process known as adult brain cytogenesis (a broader designation used to encompass both neuro- and gliogenesis), is influenced by experience [16–19]. Brain cytogenesis also serves as a pathological substrate in some neuropsychiatric disorders [3, 20–23] and is modulated by different classes of drugs, including antidepressants and antipsychotics [22, 24–27]. Such observations raised the hypothesis that these newly generated cells could participate in pivotal brain functions and led to the implementation of strategies

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to experimentally dissociate the contribution of new cells from the post-mitotic cell population, in an attempt to understand their specific role. Indeed, several studies using either Glial Fibrillary Acidic Protein (GFAP)-Thymidine kinase (Tk) [28–31] or Nestin-Tk mice lines [32, 33] that allow for specific elimination of progenitor cells upon chronic administration of anti-viral pro-drugs, such as ganciclovir (GCV), have provided evidence for the requirement of new cells for a specific subset of high-level neural processes that rely on hippocampal function. Few years ago, the first transgenic inducible rat model of cytogenesis abrogation—the GFAP-Tk rat—was generated to provide information on the relevance of adult cytogenesis for brain physiology and behavior in mammals' species beyond mice [34]. A previous general characterization of this rat model, after a total time of cytogenesis suppression of 8 weeks, showed no alterations in anxiety-like behavior, hedonia or hippocampus-dependent spatial pattern separation, a function that has been attributed to young adult-born granule neurons in the hippocampus [34]. However, a subsequent study, using a distinct transgenic GFAP-tk rat model, revealed that blockade of neurogenesis during 8 weeks induced reward-related behavioral deficits in the sucrose consumption test [35]. These studies exemplify the discrepancies found across species and testing conditions, and highlight how the field lacks a comprehensive understanding of the contribution of adult-born hippocampal cells at distinct maturational stages for brain function. Here, we used the first validated GFAP-Tk rat model [34] but adopted a distinct GCV treatment regimen, to unveil at which maturational age adult-born cells participate in distinct hippocampal-dependent functions. For that we took a multi-dimensional analysis of the specific role of immature versus mature newborn hippocampal cells, by adopting two independent experimental timelines to target new neurons' function at specific maturation time-points: a "short-term" analysis, in which behavioral tests were conducted immediately after the cessation of a 21-days treatment, to assess the role of immature cells, and a second "long-term" analysis time-point, 4 weeks post-GCV treatment cessation, at which cells were expected to have reached maturity. Moreover, as we sought to further understand the functional relevance of these cells for behavior, we conducted electrophysiological recordings of both the hippocampus and the medial Prefrontal cortex (mPFC), following cytogenesis abrogation.

Our results show a time-dependent manifestation of both emotional and cognitive impairments, as well as deficits in the hippocampus-to-PFC communication, suggesting that participation of adult-born hippocampal cells to hippocampal function depends on their maturational age.

RESULTS

Rat model and general health condition

In order to have a comprehensive view of the role of adult-born cells in the hippocampus for behavior and brain physiology, we used a GFAP-Tk rat model that allows the specific elimination of GFAP-positive proliferating cells upon treatment with the pro-drug ganciclovir (GCV; guanosine analog). In this model, the viral Tk is expressed under the control of the GFAP promoter. Upon GCV treatment, the proliferating cells uptake GCV along with other nucleotides necessary for DNA replication that are metabolized by the viral thymidine kinase, expressed in GFAP-positive cells in GFAP-Tk animals. Once GCV is metabolized by GFAP-positive proliferating NSCs, intracellular toxic by-products are formed inducing cell death. Both wt and gfap-tk +/− rats were treated with GCV for 21 days. As previous studies have shown that peripheral administration of GCV in GFAP-TK animals may cause deleterious effects on skin and intestine, all animals included in this study were weighed every 4 days and the coat state was assessed through a Coat State Score table (Supplementary Table 1), as measures of animals' wellbeing. No significant changes in the body weight gain or in the coat score were observed between experimental groups (Fig. 1a, b; Weight gain: $F_{(4,90)} = 0.1287$, $p = 0.9716$; Coat score: $F_{(5,108)} = 0.9487$, $p = 0.4528$). These measures indicate that elimination of GFAP-positive proliferating cells did not produce deleterious effects in the general wellbeing of animals.

Cytogenesis abrogation in the hippocampal dentate gyrus (DG) of GFAP-TK rats

The efficacy of the chronic GCV treatment in GFAP-Tk rats as a cytogenesis abrogation approach was tested by immunofluorescence analysis of endogenous markers and also of BrdU, an exogenous marker injected before sacrifice to follow maturating cells. To assess immature 1-week-old cells, BrdU was injected during the last 5 days of GCV treatment and rats were euthanized following a period of behavioral analysis (Fig. 1a; 7 days; short-term characterization). To assess newborn mature cells, animals were injected with BrdU during the same period, but sacrificed only 4 weeks after ending GCV treatment (Fig. 1a; long-term characterization). We analyzed hippocampal cell proliferation and survival in the dorsal and ventral DG (dDG and vDG, respectively). In the short-term analysis, GCV treatment virtually eliminated the entire population of DCX-positive neuroblasts in GFAP-Tk rats, both in the dorsal and ventral poles of the DG (Fig. 1c, d; dDG: $t_8 = 8.853$, $p < 0.0001$; vDG: $t_8 = 24.34$, $p < 0.0001$). Moreover, GCV treatment promoted a significant reduction in the generation and short-term survival of DCX-BrdU-double-positive cells in both the dDG (Fig. 1e; $t_8 = 7.941$, $p < 0.0001$) and the vDG (Fig. 1e; $t_8 = 13.79$, $p < 0.0001$). A similar analysis, conducted 4 weeks after the end of treatment, showed how GCV precluded long-term survival of newborn cells (total BrdU-double-positive cells), in both hippocampal poles (Fig. 1f–h). Specifically, both hippocampal neurogenesis and astrogliogenesis were markedly suppressed, as shown by the reduced survival of new neuronal BrdU/NeuN double-positive cells (Fig. 1f–h; dDG: $t_8 = 19.81$, $p < 0.0001$; vDG: $t_8 = 13{,}38$, $p < 0.0001$) and glial BrdU/GFAP double-positive cells (Fig. 1f–h; dDG: $t_8 = 6.782$, $p < 0.0001$; vDG: $t_8 = 4.867$, $p = 0.0006$). This latter approach allowed us to pinpoint the specific role of 4- to 7-week-old newly generated cells, while leaving the remainder population of cells intact, as DCX-positive cells could be observed in the DG at this time-point (Fig. 1i, j).

Adult-hippocampal cytogenesis abrogation at the short-term impacts only anxiety-like behavior

Immediately after the cessation of GCV treatment (Fig. 2a), GFAP-Tk animals did not present signs of anhedonic behavior in the SCT (Fig. 2b; $t_{17} = 0.4173$; $p = 0.3408$) or SDT (Fig. 2c and Supplementary Fig. 1; $t_{16} = 0.9930$; $p = 0.1677$), as shown by similar preference levels compared to wt animals. Recording of 50 kHz USVs during the SDT test, an additional measurement reflecting a positive affective state of the animals [36, 37], did not disclose significant differences between wt and GFAP-tk rats (Fig. 2d; $t_{16} = 0.1376$; $p = 0.4461$).

Moreover, cytogenesis abrogation did not produce short-term precipitation of depressive-like behavior, as both GFAP-Tk and wt animals presented similar immobility and latency to immobility times in the FST (Supplementary Fig. 2b, c; immobility: $t_{17} = 0.5286$, $p = 0.3020$; latency: $t_{17} = 0.1970$, $p = 0.4231$). In addition, we evaluated whether abrogation of NSCs had short-term effects on anxiety-like behavior. In an EPM trial, GFAP-Tk animals did not behave differently from wt animals (Supplementary Fig. 2d; $p = 0.1325$); however, in an NSF task, a test paradigm that involves a more pronounced neophobic context, short-term suppression of brain cytogenesis was sufficient to elicit significantly higher latency to feed periods, in relation to wt animals (Fig. 3e; $t_{17} = 6.202$, $p < 0.0001$); no differences were found in appetite drive (Supplementary Fig. 2e; $t_{17} = 0.6487$, $p = 0.263$) that could undermine results interpretation in this task. At this time-point, animals

were also tested in a spatial reference memory task and we found no effect of cytogenesis abrogation in animals' performance (Fig. 2f). In summary, short-term depletion of newly generated immature brain cells was correlated with the precipitation of a mono-dimensional anxiety-like behavioral phenotype in the NSF test (Fig. 2g).

Deficits in hedonic behavior emerge 4 weeks after adult brain cytogenesis abrogation

In addition to the previous characterization of the behavioral relevance of newborn immature brain cells, we evaluated the long-term behavioral profile of adult rats, 4 weeks after cessation of GCV treatment. (Fig. 3a). In this time-point, GCV treatment

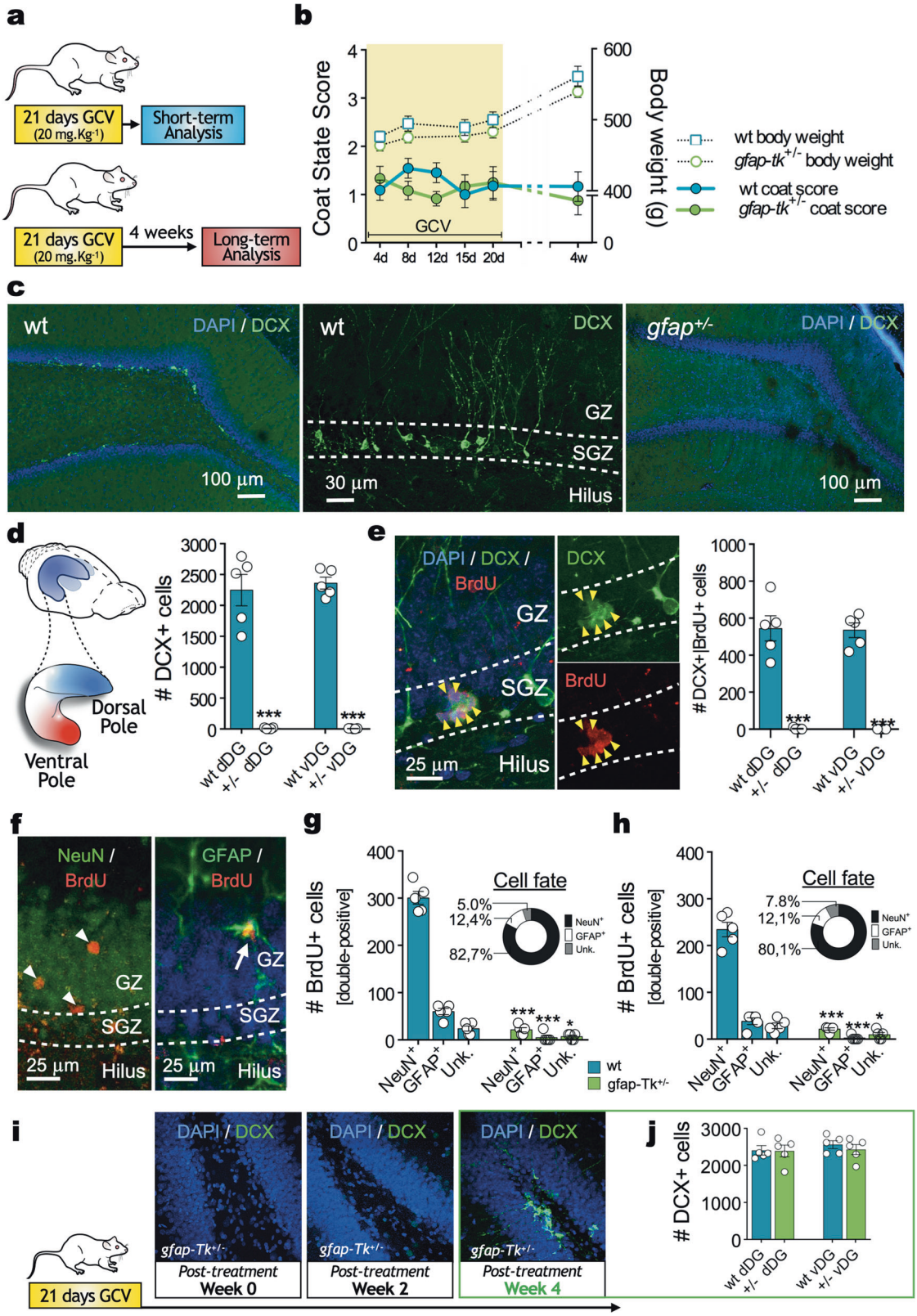

Fig. 1 Short- and long-term cell generation and survival in the ventral and dorsal dentate gyri, following GCV treatment. a Depiction of the experimental timeline for the "short-term analysis" (behavioral tests conducted immediately post-abrogation) and the "long-term analysis" (behavioral analysis conducted 4 weeks post-abrogation). **b** General health indicators (body weight and coat state) of wild-type (wt) *versus* gfap-tk heterozygous rats (*gfap-tk* +/−). **c** Left and middle panel showing low- and high-magnification images, respectively, of doublecortin (DCX)-positive neuroblasts in the DG of wt animals. GCV treatment virtually eliminated all DCX cell population, on *gfap-tk* +/− rats (right panel). **d** DCX + cell counts following GCV treatment, in dorsal (dDG) and ventral dentate gyri (vDG). **e**–**h** BrdU was administered at the end of GCV treatment to perform cell survival and cell fate analysis. Short-term survival of young neuroblasts (**e**, left panel) was assessed, following GCV treatment. Four weeks post BrdU injection, neurogenesis (**f**, left panel) and astrogliogenesis (**f**, right panel) were analyzed. GCV treatment compromised long-term differentiation and survival of newborn cells, both in dDG (**g**) and vDG (**h**). **i** Representative confocal images of the hippocampal neurogenic niche immediately after the end of ganciclovir (GCV) treatment (left panel) and at 2 (middle panel) and 4 weeks (right panel) after the end of GCV administration. Four weeks after cytogenesis ablation doublecortin-positive (DCX) cells can be observed in the DG. Magnification = 200x. **j** DCX + cell counts 4 weeks after the end of GCV administration, in dorsal (dDG) and ventral dentate gyrus (vDG). At this time-point, no differences were observed in the number of DCX-positive cells between gfap +/− animals versus wt animals. Error bars denote s.e.m. *$p < 0.05$, ***$p < 0.001$; $n = 5$ per group. GCV ganciclovir; SGZ subgranular zone; GZ granular zone; unk. unknown cell phenotype.

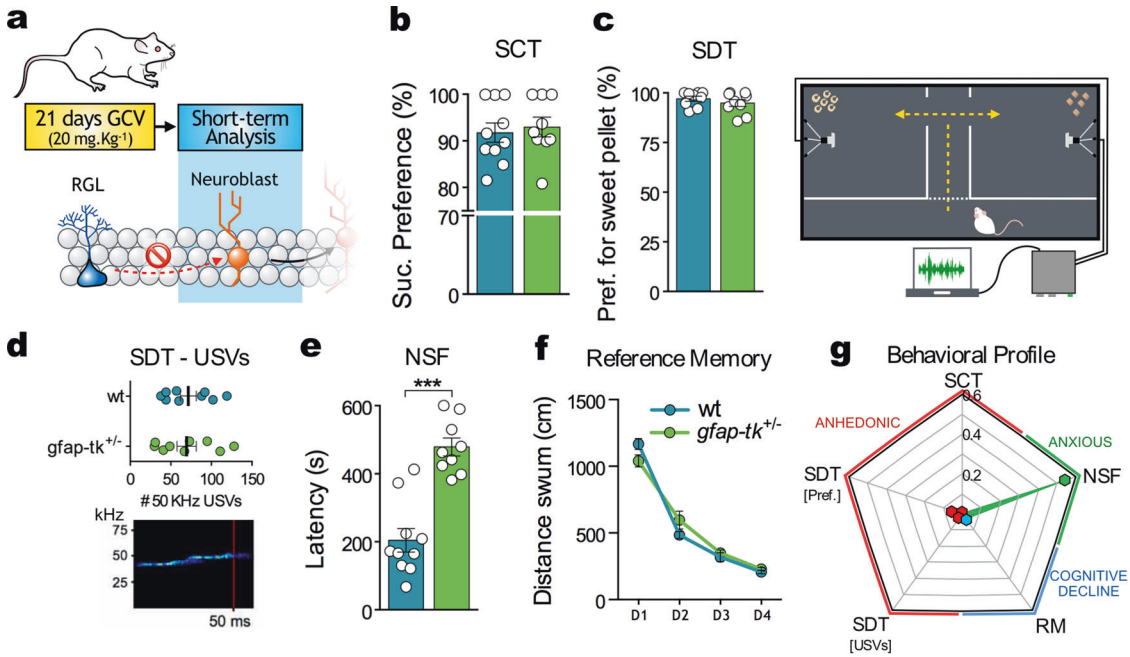

Fig. 2 Short-term behavioral effects of adult brain cytogenesis abrogation. a In this experimental set, different behavioral analyses were conducted immediately after GCV administration. **b**, **c** Anhedonic behavior was measured in the (**a**) SCT and in the (**b**) SDT. **d** During SDT trials, 50 KHz ultrasound vocalizations (USVs) were quantified. **e** NSF paradigm was used to measure anxiety-like behavior. **f** Cognitive performance was evaluated in a spatial reference memory task. **g** Radial graphical representation of multiple measures of gfap-tk +/− animals performance in different behavioral tasks. Different behavioral dimensions were assessed: anhedonic behavior, anxiety-like behavior, and cognitive performance. Plotted values are normalized to the average value of the control group; data points that are further away from the center (zero) refer to significant deficits in the corresponding behavioral task. Shortly after brain cytogenesis depletion, animals presented a mono-dimensional anxiety-like phenotype. Error bars denote s.e.m. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 9$–10 per group. GCV ganciclovir; RGL radial-glial cells; SCT sucrose consumption test; SDT sweet drive test; NSF novelty suppressed feeding; RM reference memory.

elicited the development of anhedonic behavior, manifested as a decreased preference for sucrose in the SCT (Fig. 3b; $t_{17} = 3.781$, $p = 0.0007$), as well as decreased preference for sweet pellets in the SDT (Fig. 3c and Supplementary Fig. 3; $t_{17} = 3.602$, $p = 0.0011$). These results were further corroborated as GFAP-Tk animals presented a decreased number of 50 kHz positive USVs in the SDT (Fig. 3d; $t_{15} = 1.806$, $p = 0.0455$). As observed in the short-term behavioral characterization, no differences were observed in the FST immobility (Supplementary Fig. 4b; $t_{18} = 0.2709$, $p = 0.3948$) and latency to immobility time (Supplementary Fig. 4c; $t_{18} = 1.232$, $p = 0.1168$) or in a water maze reference memory task (Fig. 3f). On the other hand, anxiety-like deficits in the NSF test were still observed in this time-point (Fig. 3e and Supplementary Fig. 4d; $t_{17} = 2{,}511$, $p = 0.0112$). Taken together with the previous short-term behavioral characterization, these results highlight a time-dependent manifestation of multi-dimensional behavioral deficits, following brain cytogenesis abrogation. In particular, the lack of newly formed mature (4 to 7-week-old) neuronal and glial cells induced anhedonic behavior (Fig. 3g), which becomes super-imposed to the early installed anxiety-like phenotype.

Brain cytogenesis abrogation produces alterations in cognitive strategies

Our results show that abrogation of adult brain cytogenesis did not produce significant alterations in the distance to reach the escape platform during the learning phase of the spatial reference memory task in the water maze, both in short- and long-term analyses (Figs. 2f and 3f). Following the reference memory task, the platform was repositioned in the opposite quadrant to test behavior flexibility (Fig. 4a). In this task, GFAP-Tk animals spent proportionally less time in the new quadrant, compared to wt rats (Fig. 4a; $t_{14} = 0.4640$, $p = 0.0002$). Interestingly, this

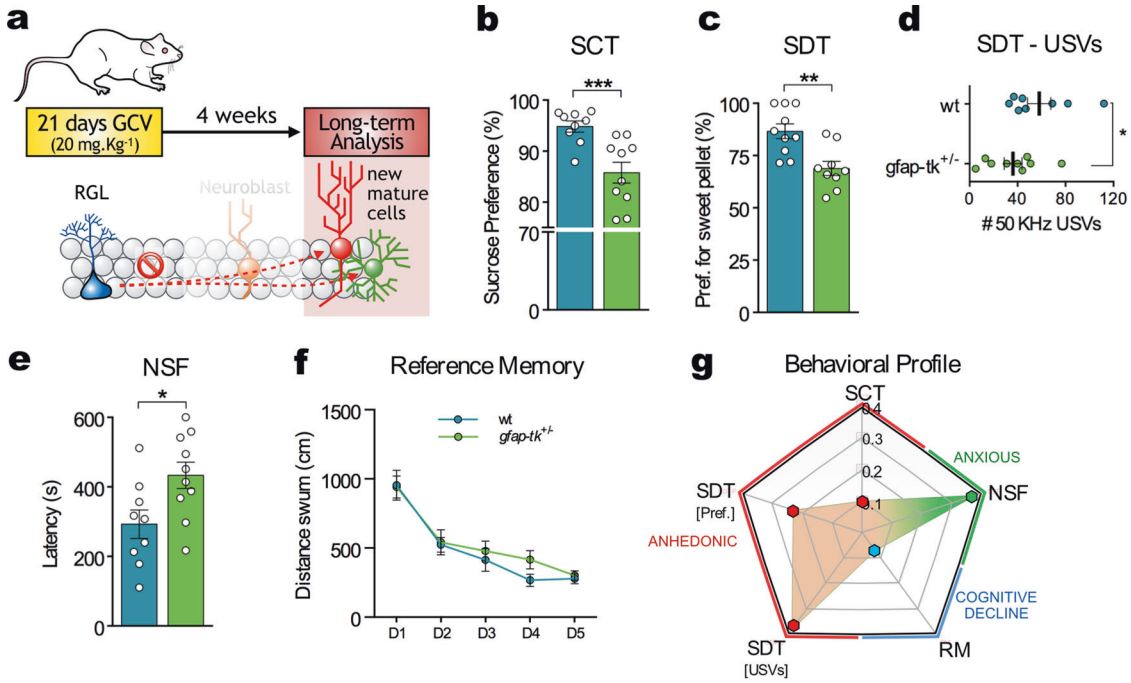

Fig. 3 Long-term behavioral effects of adult brain cytogenesis abrogation. a Behavioral analysis were conducted 4 weeks following GCV administration in an independent experimental set. **b-c** Anhedonic behavior was measured in the (**b**) SCT and in the (**c**) SDT. **d** During SDT trials, 50 KHz ultrasound vocalizations (USVs) were quantified. **e** NSF paradigm was used to measure anxiety-like behavior. **f** Cognitive performance was evaluated in a spatial reference memory task. **g** Lack of 4-week-old newborn cells produced multi-dimensional behavioral phenotype changes. Error bars denote s.e.m. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; $n = 9–10$ per group. GCV ganciclovir; RGL radial-glial cells; SCT sucrose consumption test; SDT sweet drive test; NSF novelty suppressed feeding; RM reference memory.

behavioral deficit was also evident, at a lesser extent, when tested immediately after cytogenesis abrogation (Supplementary Fig. 5a, b).

We also assessed working memory in a water maze test paradigm, 4 weeks after cessation of GCV treatment, in which the location of the escape platform was repositioned every day in a new quadrant. The ability of animals to adapt and retain the new platform position in each trial was assessed. Abrogation of cytogenesis in GFAP-Tk rats produced long-term deficits in this cognitive domain, reflected as a significant increase in the overall distance traveled to reach the escape platform (Fig. 4b).

Furthermore, we analyzed cognitive strategies adopted along the trials to reach the escape platform (Fig. 4c–f). This analysis showed that GFAP-Tk rats are able to reach test performances comparable to wt controls at the expense of using predominantly non-hippocampal-dependent strategies ("Block 1") (Fig. 4c–f). In fact, these animals delay the transition from non-hippocampal-dependent ("Block 1") to hippocampal-dependent strategies ("Block 2") (Fig. 4c–f): >50% of wt animals enter Block 2 strategies by test day 2, while GFAP-Tk rats do so mainly on days 3 and 4, resulting in an increased mean duration of Block 1 in relation to wt rats (Fig. 4e, f; Block 1: $t_{15} = 4.082$; $p = 0.0005$; Block 2: $t_{15} = 3.113$; $p = 0.0036$). This cognitive adaptation occurs more pronouncedly at the long-term analysis time-point, although immediately after cytogenesis abrogation, mild deficits were already found in these navigational patterns and in working memory between the tested groups (Supplementary Fig. 5c–f).

Adult brain cytogenesis abrogation impairs contextual fear- but not cued-conditioning

To further explore the long-term hippocampal-dependent cognitive deficits observed in the Water Maze, we assessed associative learning and memory using the contextual fear-conditioning paradigm, 4 weeks after cessation of GCV administration. Animals were submitted to a context probe, aimed to test hippocampal-dependent memory consolidation, and a light-cued probe, aimed to assess the integrity of memory circuits relying to a lesser extent in hippocampal function (Fig. 4g). First, we assessed whether both experimental groups presented similar percentages of freezing after the light-shock pairings, so that potential divergent values in this parameter would not undermine a clear interpretation of the results obtained in the following trials. In fact, after the conditioning trials both groups had increased average freezing percentages (Fig. 4h; Cue-shock association: $F_{(1,36)} = 237.2$, $p < 0.0001$), which were similar between them; (Fig. 4h; Group effect: $F_{(1,36)} = 0.0008$, $p = 0.9295$). In the context probe, GFAP-Tk animals presented a reduction in the percentage of freezing when exposed to a familiar context, compared to wt animals (Fig. 4i, context A; $t_{18} = 1.866$; $P = 0.0196$). Switching to a novel environment, decreased freezing times in similar fashion for both groups (Fig. 4i, context B; $t_{18} = 1.303$; $p = 0.2089$). In the light probe, both groups presented similar percentages of freezing after exposure to the light cue (Fig. 4j; $t_{18} = 0.8273$; $p = 0.4189$). Overall, CFC results show that GFAP-Tk rats display long-term specific deficits in contextual hippocampal-associated memory, while associative extra-hippocampal-dependent memory remains intact.

Adult cytogenesis abrogation produces synaptic plasticity impairments affecting dHIP-mPFC communication

In order to assess the mechanism through which lack of 4–7-week-old adult-born hippocampal cells could drive such behavioral impairments, we sought to assess inter-regional synaptic plasticity. For that, we performed a functional characterization of the hippocampus-to-PFC network in an independent set of rats. First, we analyzed the electrophysiological properties of Local Field Potentials (LFPs; oscillations that result from coordinated rhythmic activity of neuronal populations) in these areas (Supplementary Fig. 6). The analysis of the individual LFP power on specific frequency bands, namely at theta, beta and low gamma, provides an estimate of the network activity amplitude for each frequency

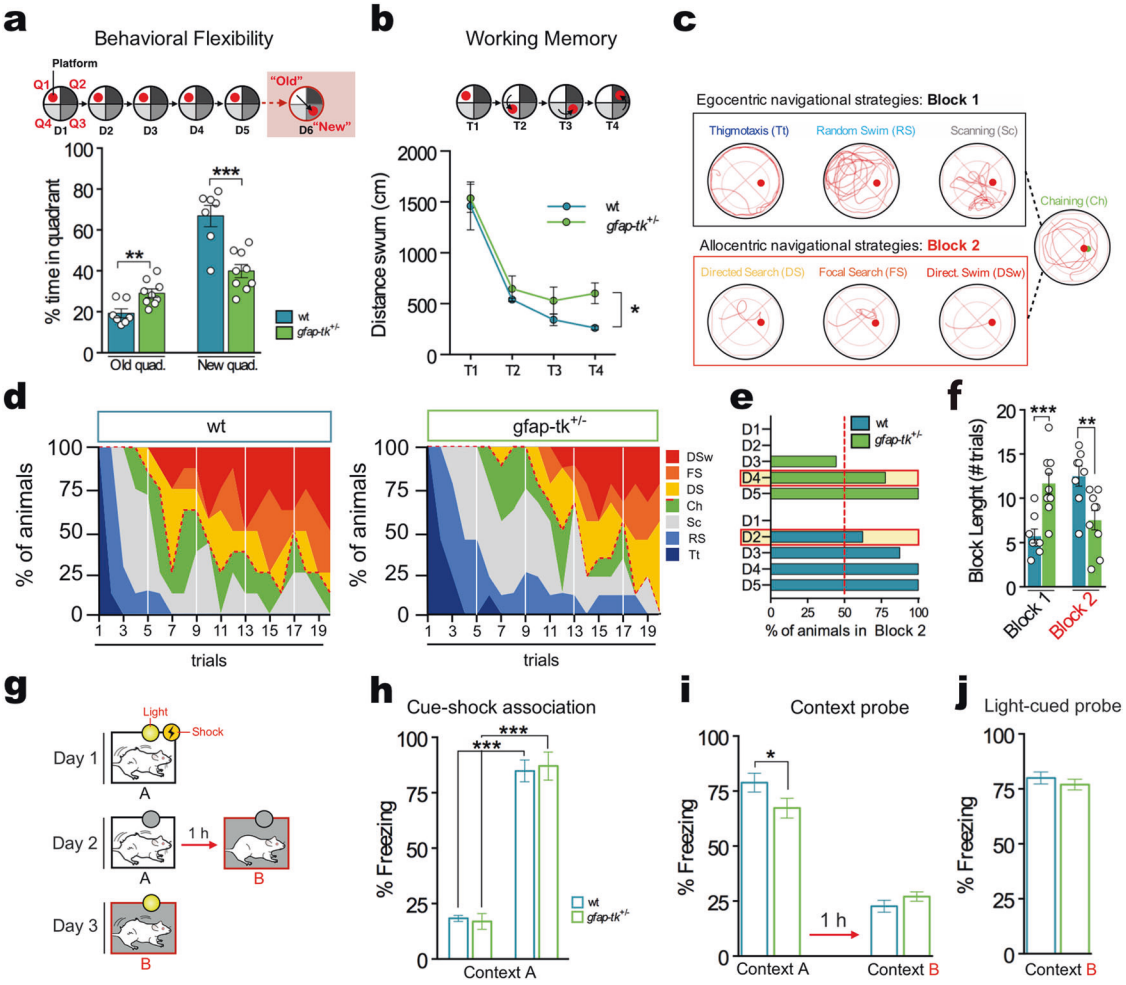

Fig. 4 Long-term cognitive behavior characterization. a Reversal learning task. **b** Working memory task in the water maze. **c**, **d** Schematic representation and color code for each strategy (**c**) and the average prevalence of each strategy by trial number are shown (**d**) both for wild-type (wt) and for gfap-tk +/− animals. **e** Distribution of strategies-block boundaries (Block 1: "non-hippocampal-dependent strategies"; Block 2: "hippocampal-dependent strategies"). **f** Graphical comparison of overall block length. **g**–**j** Contextual fear-conditioning (CFC) task. Freezing behavior was assessed following cue-shock pairing (**h**), as well as in context-associated (**i**) and light-cued trials (**j**). Overall, lack of 4-weeks-old newborn cells promotes the manifestation of long-term mnesic and behavioral flexibility deficits. Error bars denote s.e.m. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; $n = 9$–10 per group.

in baseline conditions. Additionally, we performed coherence measurements between simultaneously recorded LFPs (Fig. 5), as a measure of phase and amplitude synchronization between the recorded regions. No differences were found between wt and GFAP-Tk groups in the LFP power at theta, beta and low gamma frequencies in any of the regions (Supplementary Fig. 6**)**. Contrastingly, the coherence measurements between the mPFC and the dHIP were significantly decreased in the theta band in GFAP-Tk rats compared to wt (Fig. 5a; $t_8 = 2.542$; $p = 0.0346$), suggesting compromised communication between these two brain regions. No significant differences were found in the coherence between mPFC and vHIP (Fig. 5b).

DISCUSSION

Recognition of the mammalian brain ability to produce new cells throughout adulthood has added an important layer of complexity to the way neural plasticity is perceived. Importantly, during the last few decades, a significant body of evidence supported the occurrence of adult neurogenesis not only in rodent models but also in the human brain, namely in the hippocampus [2–5].

Three major types of strategies have been adopted to abrogate adult cytogenesis and study its impact on brain homeostasis: i) the use of x-ray irradiation [29, 30, 38, 39], which is a regionally restricted method, but lacks cell-specificity, affecting also mature cells and eliciting significant inflammatory response; ii) the use of cytostatic drugs [22, 40, 41], which abrogates cytogenesis in all cytogenic niches of the brain, but that again does not spare other mitotic cell populations and may trigger dose-dependent systemic effects, and, iii) the use of transgenic lines [28–31, 33, 35, 42–45] that allow for the specific (promoter-driven) elimination of NSCs, without interfering with other cell types or having major general effects. This last approach has been proven useful to elucidate the neurobiological role of cytogenesis in the adult brain. Nevertheless, the dynamics of adult cytogenesis, as well as the role of newly generated cells at specific maturation stages for brain function and behavior, are far from being completely understood, with apparently conflicting data arising from the use of different animal models and experimental timeframes. Time is indeed a fundamental variable to bring into the equation, as both newly generated neurons and astrocytes, depending on their maturational stage, may fulfill different functions according to their properties and ability to respond to specific stimuli. In addition, it is also important to consider that most data in the field come from mouse models and very little is known about how conserved their functional relevance might be throughout species. Thus,

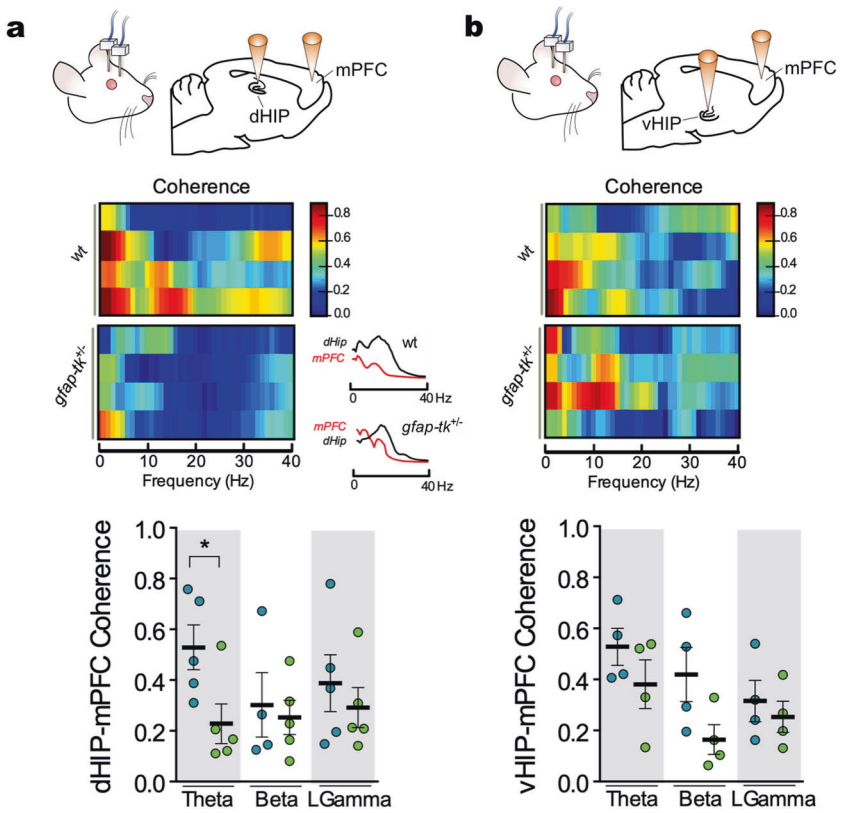

Fig. 5 Adult-hippocampal cytogenesis abrogation induces long-term decrease in spectral coherence between the dorsal hippocampus (dHIP) and the medial prefrontal cortex (mPFC). Spectral coherence was assessed between dHIP-mPFC (**a**) and vHIP-mPFC (**b**). Middle panels show spectrograms, in which each horizontal line in the *y*-axis represents the spectrogram of an individual rat (four representative animals from each group are shown). Group comparison of the coherence values for each frequency band are presented on the bottom plots. Error bars denote s.e.m. $*p < 0.05$. $n = 4–5$ per group.

extending these analyses to other mammalian models, namely rats, which have been shown to present distinct properties regarding adult-hippocampal cytogenesis [46–49], may provide comprehensive data on the functional relevance of mammalian adult cytogenesis.

In this work, we have used one of the first GFAP-Tk transgenic rat models developed [34] to specifically eliminate GFAP proliferating stem cells and, as a consequence, abrogate the generation of new cells in the adult brain. In this model, Tk expression was only found in GFAP-expressing cells which were also Sox2-positive, indicating that Tk expression was restricted to neural stem cells [34]. The used GCV-administration protocol allowed for an almost complete abrogation of hippocampal cytogenesis, with no detectable BrdU cells in GCV-treated animals, and a residual DCX-positive population ranging from 0% to 1.06% of the average value observed in control animals. Although the contribution of this extremely small population of neuroblasts to the reported behavioral results cannot be excluded, we were able to achieve an abrogation of over 99% of proliferative cells and this effect was stably maintained until at least 2 weeks after treatment cessation (Supplementary Fig. 1). Comparable rates of neurogenesis abrogation were previously reported [34, 35]. Previous studies using GFAP-Tk mouse models have reported changes in the coat condition and weight loss upon GCV administration. Here, we did not observe significant changes in any of these parameters throughout the GCV treatment period, in accordance with previous studies using this and other GFAP-Tk rat models [34, 35], which may be explained as a species-related or dosage-related effect. Our approach was effective to abrogate hippocampal cytogenesis, compromising the generation of new neurons, but also of new astrocytes, which we found to correspond roughly to 12% of newborn cells in the hippocampal DG. Although the generation of new astrocytes from GFAP-positive NSCs has been shown to occur to a much lesser extent than neurogenesis in the adult hippocampus [14, 50], we cannot rule out the possible contribution of these newborn astrocytes for the behavioral and functional findings reported herein.

Considering that adult-born hippocampal cells display different structural and functional properties throughout their development, we assessed the role of either immature or mature newborn cells, by adopting two experimental timeframes: a *short-term* analysis, to assess the functional importance of immature newly generated cells (whose production has been halted during GCV treatment), and a "long-term" time-point to assess the function of 4- to 7-week-old cells (new cells that would have been mature at the time of testing).

Similarly to what has been previously shown in another rat model [41] or using GFAP-Tk mice [28], short-term abrogation of cytogenesis in GFAP-Tk rats produced specific behavioral deficits, namely on anxiety-like behavior, in the NSF test but not in the EPM. Interestingly, in previous studies using GFAP-Tk mice [28], heightened anxiety-like behavior was only evident when heterozygous mice were exposed to stress conditions; therefore, it is likely that the test environment of the NSF task, with a brightly illuminated center of the arena, poses a more aversive and neophobic environment to the tested animals, in opposition to the dimly illuminated environment of the EPM apparatus. The more aversive context of the NSF trials may trigger a stress-like response, sufficient to elicit anxiety traits. Indeed, abrogation of NSCs in the adult rodent brain has been shown to produce imbalances in glucocorticoid signaling, under acute stress conditions, resulting in an exacerbation of the stress response

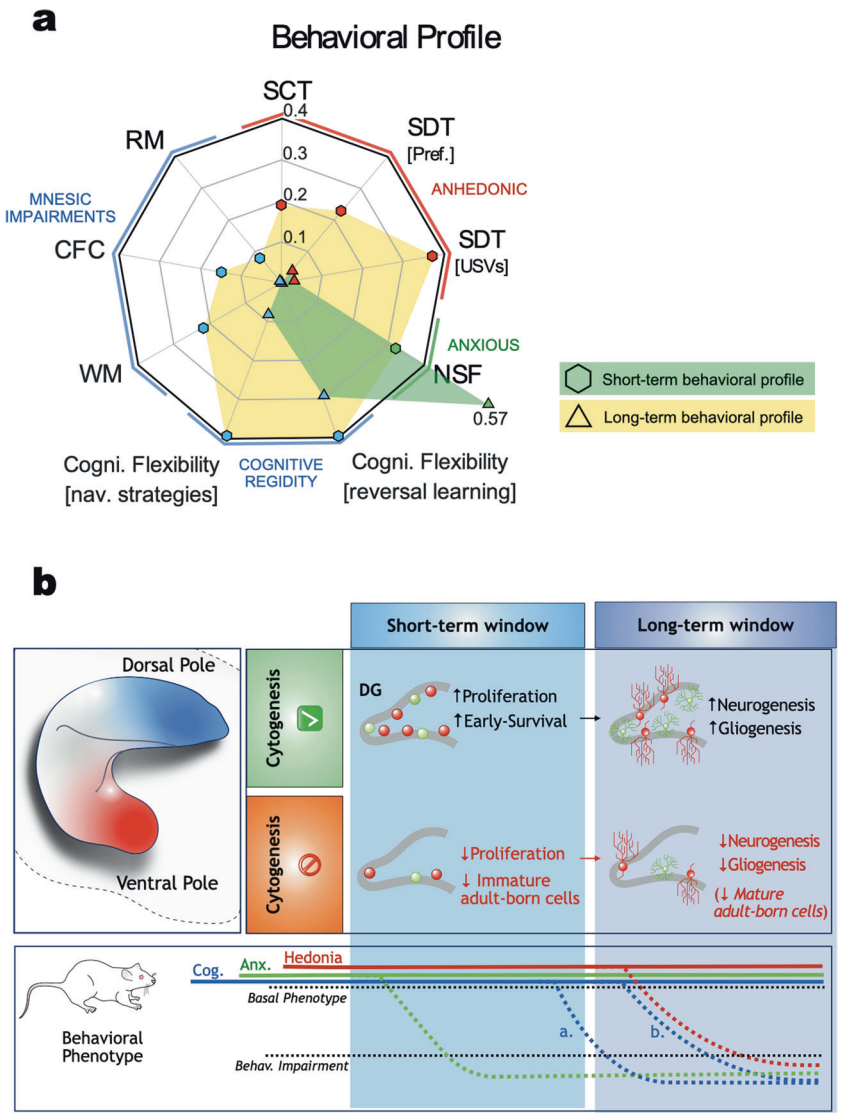

Fig. 6 Time-dependent functional correlates of adult brain cytogenesis. a Radial graphical representation of the behavioral phenotypes upon short-term *versus* long-term abrogation of adult brain cytogenesis. **b** While loss of newly generated immature hippocampal cells precipitates anxiety-like behavior (dashed green line), and mild loss of cognitive flexibility (dashed blue line "**a**"), the lack of 4-week-old newborn neurons and astrocytes is associated with long-term development of multi-dimensional deficits in multiple additional cognitive functions (dashed blue line "**b**"), anxiety control and hedonic behavior (dashed red line).

[28, 51, 52]. Accordingly, previous works have additionally shown how fluctuations in endogenous glucocorticoids and glucocorticoid receptor expression levels control activation of NSCs and precursor cells and could produce pathological consequences on neuronal integrity and, hence, on hippocampus-dependent functions [53, 54]. These findings shed light on why specific behavioral deficits reported herein only emerge under aversive test environments.

Contrastingly, long-term abrogation of hippocampal cytogenesis triggered, in addition to hyperanxiety, anhedonic behavior, accompanied by a decreased number of positive 50 kHz USVs in the SDT test. We have previously shown how the combined analysis of both food preference and the number of 50 KHz USVs in the SDT provides an invaluable tool to accurately characterize anhedonic behavior in animals chronically exposed to stress [37]. Moreover, quantification of 50 KHz USVs during this task was a highly sensitive measure to discriminate animals that responded to treatment from non-responder animals. These results suggest a time-dependent participation of adult cytogenesis in the modulation of different emotion-related behavioral dimensions, supporting a much more complex view of the neurogenic hypothesis of depression [55] (Fig. 6). In line with previous studies using distinct models [31, 32, 34, 39], we found that the overall performance of GFAP-Tk rats in a spatial reference memory task was not changed after cytogenesis abrogation. However, here, we also analyzed the cognitive strategies employed to reach the escape platform in the water maze, as they have been suggested to depend on adult-hippocampal cytogenesis [56] and found that the lack of 4- to 7-week-old cells compromise transition from non-hippocampal dependent to hippocampal-dependent strategies to find the platform in spatial reference memory tasks. Moreover, we found behavioral flexibility and working memory to be also affected at this time-point, a result that may apparently contrast with data previously obtained using this animal model in the radial maze [34]; however, it is important to highlight how this discrepancy may well be related with the cytogenesis abrogation timeline used or the distinct stress conditions of the tests [57]. In fact, similarly to the strategy used in previous mice studies, Groves et al. administered GCV during an 8-week period and conducted behavioral experiments immediately after brain cytogenesis

abrogation. That approach allowed sustained elimination of NSCs for 8 weeks, with two important implications at the time of behavioral testing: (i) the niche lacked simultaneously older newborn hippocampal cells, as well as more recently generated immature neuroblasts and (ii) this long-term sustained hippocampal cytogenesis blockage could allow the development of compensatory mechanisms to overcome cognitive and emotion-related deficits. In the present work, we restricted the cytogenesis abrogation time-window, by interrupting GCV treatment after 21 days and allowing recovery of the generation of neuroblasts and new astrocytes from unaffected quiescent NSCs. In fact, 4 weeks after the end of GCV treatment, the neurogenic niche was repopulated while the number of newly generated 4–7-week-old cells, both neurons and astrocytes, was decreased. Hence, by using this approach, we were able to tackle functional properties specifically of mature newborn neurons and/or astrocytes, while not interfering with the generation of new immatures cells at the time of behavioral and electrophysiological testing. Interestingly, and in accordance with the concomitant above-mentioned deficits on mood and anxiety-like behavior following long-term cytogenesis abrogation, a link has been suggested between the role of neurogenesis for cognitive flexibility and the development of emotional deficits [57]. In support of this hypothesis, cognitive flexibility is impaired in many psychiatric disorders that present overlapping emotional deficits. Moreover, in experimental models, cytogenesis abrogation promotes decreased cognitive flexibility that impairs ability to adapt to novel non-aversive environments. Finally, in line with this, and as previously reported [32, 39, 58], we observed that the lack of mature newborn cells produced impairments in contextual fear conditioning. Overall, our results show that most behavioral alterations caused by an anti-cytogenesis insult come to light after 4 weeks, when newborn cells, that had been otherwise generated within the hippocampus were expected to be fully functional and integrated in the pre-existing neuro-glial network. Interestingly, these results are in line with a previous study reporting no alterations 1 week following cytogenesis abrogation, but unveiling the development of cognitive deficits when the analysis was conducted 2 and 4 weeks post abrogation. More so, Gu and colleagues [12] showed how using optogenetics to interfere with the function of 4-weeks-old new neurons, but not younger cells, elicits the development of impairments in contextual fear memory and in cognitive strategies used in the water maze task. The same authors did not find 8-week-old new cells to participate in these behavioral domains. Although we did not abrogate cells older than 7-weeks, it is also important to consider the animals' species (rat versus mice) and age (adult versus young adolescent) as potential confounding factors when trying to conciliate our results with those of Gu et al. [12].

Adult-born neurons between 2 to 6 weeks following neuronal birth exhibit distinct properties from their mature counterparts, namely hyperexcitability and enhanced synaptic plasticity [12], which may provide them an advantage in the competition for synaptic connectivity, and make them particularly suitable for information processing [59–61]. Previous studies have shown how abrogating a small population of newborn hippocampal cells could produce significant impairments in the intrinsic hippocampal circuitry, particularly the DG LTP and LTD response to perforant path input [31, 39, 62], as well as to other links to other brain regions, namely to the mPFC [63, 64]. Of particular importance for the latter, it is known that during spatial working memory tasks, neural activity in the mPFC becomes synchronized with theta oscillations in the dorsal hippocampus, and the strength of hippocampal–prefrontal synchrony is correlated with behavioral performance [63]. Interestingly, our results show, for the first time, that cytogenesis abrogation compromises the synaptic communication between the dorsal hippocampus and the mPFC, as suggested by the significantly decreased synchrony between oscillations in these regions, including in the theta band. Neural oscillations, namely those in the theta band, are central for information processing, both within the hippocampal circuit and in its association to cortical regions [65]. Thus, we propose that these deficits on synaptic plasticity and inter-regional communication may account for the observed cognitive deficits in behavioral flexibility and spatial reference memory [66, 67]. While the available literature suggests that hippocampal theta oscillations may be involved in the exercise-driven potentiation of DG neurogenesis [68, 69], the net contribution of newly formed neurons to theta oscillations and to the theta-mediated entrainment of cortical regions remains unknown. Our current findings are in line with Nokia and colleagues (2012) in that the long-term treatment with the cytostatic temozolomide reduced neurogenesis, impacted on endogenous theta activity, and disrupted theta-related cognitive behavior [70]. Moreover, we have previously shown that the deletion of AP2γ, a modulator of adult-hippocampal glutamatergic neurogenesis, reduces hippocampal neurogenesis, and leads to reduction of theta coherence and behavior deficits dependent on the HIP-PFC connectivity [71]. This suggests that the disruption of DG neurogenesis, sitting at the top of the tri-synaptic loop that generates the theta oscillations upon septal and entorhinal rhythmic inputs, should influence this essential rhythm and its basal synchronization to partner regions to sustain behavior. The basal theta coherence appears as pivotal in our data, which is in accordance with our previous studies employing chronic stress protocols. These protocols are known to reduce the levels of hippocampal neurogenesis and result in similar behavioral deficits that are dependent on cortico-limbic circuits [64, 71–73].

On the other hand, no statistically significant changes in the vHip to mPFC coherence (which represent the strongest link between the hippocampal formation and the PFC [74]) were found between Wt and GFAP-Tk rats. One explanation for this lack of effect may sit on the differences in the maturational stage of the target neurons. In fact, at least in rats, the new neurons in the ventral DG maturate later than those in the dorsal DG [48], which may be determinant for their functional relevance within the HPC-mPFC circuit. In the future, it would be of interest to run these electrophysiology experiments in the short-term time-point.

In conclusion, our work supports the view on how adult brain cytogenesis participates in a subset of hippocampal-related neural processes, in a time-dependent manner. Indeed, we show how newborn neurons and astrocytes display different functional roles throughout different maturation stages, namely in cognitive and emotion-related domains (Fig. 6), The interference of adult cytogenesis in the hippocampus-to-PFC communication is a likely mechanistic link between newly generated cells and behavioral outputs. More so, by demonstrating how interfering with adult neuro- and gliogenesis produces different behavioral phenotypes depending on the experimental timeline, this work establishes common ground to reconcile contrasting results that have marked the field over the last years. Indeed, and among other confounding factors, those apparent data discrepancies may very well be a matter of time.

ONLINE METHODS

Animals

A GFAP-Tk transgenic rat line was generated, as previously described [34]. Two female GFAP-Tk founders were used to establish an in-house colony, as TK male rats were previously shown to be infertile, and thus breeding is restricted to female heterozygous rats and Wild-type (wt) males. Genotyping identified the transgenic pups and wt littermates that were used in this study. Animals were grouped-housed in polypropylene cages (2 per cage) under 12 h light: 12 h dark cycles, 22 °C, relative humidity of 55% and with food and water ad libitum.

All procedures were carried out in accordance with EU Directive 2010/63/EU guidelines on animal care and experimentation and were approved by the University of Minho Subcommittee of Ethics for the Life and Health Sciences (SECVS068/2017).

Ganciclovir treatment and experimental timeline

Ganciclovir (GCV) (Kemprotec, UK) was prepared in hydroxyethilcellulose at 30 mg/ml. Thirty (30) mg/kg were injected intraperitoneally (i.p.) once a day, for 21 consecutive days, in 3 month-old animals. Both GFAP-Tk ($n = 19$) and littermate wild-type controls (wt; $n = 20$) were divided in two subgroups and subjected to behavioral testing either in the day following GCV treatment cessation ("short-term analysis"), or 4 weeks after the end of treatment ("long-term analysis"). All animals were weighed every 4 days and the coat state (general appearance/grooming, presence of porfirin in nose, eyes and coat) was assessed through a Coat State Score table (Supplementary Table 1), as measures of animals' wellbeing. 5-Bromo-2-deoxyuridine (BrdU; B5002, Sigma-Aldrich), a thymidine analog that incorporates in the S-phase of the cell cycle, was injected daily (BrdU was diluted in 0.9% NaCl at a concentration of 0.1 g/mL. Injection volume was then adjusted to animal weight in order to achieve a daily administration of 50 mg/kg, i.p.) in all animals, during the last 5 days of GCV treatment. At the time-point of BrdU injection, cytogenesis abrogation is expected to be maximal [35].

Behavioral analysis

For the short-term experiment, all behavioral testing was conducted during a 10-days period. In the long-term experiment, additional cognitive modalities have been assessed and, therefore, behavioral testing was conducted during a 16-days period.

Sucrose consumption test (SCT)

Baseline sucrose preference values were established during a 1-week habituation period (three independent trials, every 48 h; 1 week prior the beginning of GCV treatment) during which animals were presented with two pre-weighed drinking fluid bottles, containing water or 2% (w/v) sucrose. After habituation, one single SCT trial was performed in each established analysis subgroup ("short-term" and "long-term"). Before each trial, rats were food- and water-deprived for 12 h (from 10 a.m. to 10 p.m.; during their inactive period) and exposed to the test drinking solutions for 1 h. Sucrose preference was calculated as described previously [75].

Sweet drive test (SDT)

The SDT test was used as an additional measure of anhedonic behavior [37]. Each animal was first habituated to the empty SDT chamber for 8 min. Forty-eight hours later, each animal was allowed to explore the SDT box for 10 min, where sweet (Cheerios, Nestlé®) or regular pellets (Mucedola 4RF21-GLP) were available. Preference for sweet pellets was calculated as: Preference for Sweet Pellets = Sweet pellet consumption/(Sweet pellet + regular Chow consumption) × 100. The number of entries into each chamber was used as a measure of exploratory behavior.

Ultrasonic vocalizations (USVs)

Ultrasound microphones (CM16/CMPA, Avisoft Bioacoustics) sensitive to frequencies of 10–200 KHz were placed in the SDT middle chamber, 15 cm above the floor, in order to record rats' USVs during the SDT trial. The microphones were connected via an Avisoft UltrasoundGate 416H (Avisoft Biocoustics) to a computer. Vocalizations were recorded using the Avisoft Recorder (version 5.1.04) with the following settings: sampling rate: 250,000; format: 16 bit. All 50 KHz vocalizations, identified by automated data processing, were individually analyzed and validated by a trained experimenter. The total number of 50 KHz vocalizations emitted is presented.

Novelty suppressed feeding

Anxiety-like traits were further assessed through the NSF paradigm. After a 18-h period of food-deprivation, animals were placed in an open-field arena, as previously described [22] where a single food pellet was positioned in the center. After reaching the pellet, animals were individually returned to their home cage, where pre-weighted food was available, and were allowed to feed during 10 min. The latency to feed in the open-field arena was used as an anxiety-like behavior measurement, whereas the food consumption in the animal home cages provided a measure of appetite drive. No differences were observed in the appetite drive between the experimental groups that could lead to a misinterpretation of the results (Supplementary Figs. 3c and 4b).

Elevated-plus maze. Anxiety-like behavior was examined through the elevated-plus maze (EPM) test, in a 5-min session, as previously described [22]. The percentage of time spent in the open-arm was used as an index of anxiety-like behavior.

Water maze tests

The water maze was used to test the performance in both spatial reference memory and working memory tasks, as described previously [22]. Briefly, these tests were conducted in a circular black pool (170 cm diameter) filled with water at 22 °C to a depth of 34 cm in a room with extrinsic clues (triangle, square, cross and horizontal stripes) and dim light (light intensity adjusted to 90–100 lux). An invisible platform was placed in one of four quadrants. Trials were video-captured by a video-tracking system (Viewpoint, Champagne au mont d'or, France). Animals were habituated to the test room, during the two days preceding the tests, being kept in the room for 1 h each day.

Working memory task. The working memory task was used to evaluate the cognitive domain that relies on the interplay between the hippocampal and PFC functions. An escape platform was placed in one of the quadrants and was maintained in the same position during the four daily trials. The test was performed for four days, and in each day the platform was repositioned in a new quadrant in a clockwise-fashion. In each of the daily trials animals were positioned in a different starting point (north, east, west and south) and a trial was considered as concluded when the platform was reached within the time limit of 120 s. The time of escape latency and the path described to reach the platform (distance swam) were recorded for each trial.

Reference memory task

This task allows assessing hippocampal-dependent cognitive function. For five days the platform remained on the same quadrant and animals were tested in four daily trials according to the same procedure previously described for the Working Memory Task. At the fifth day, the behavioral flexibility performance of the animals, a prefrontal cortex-dependent function, was tested by positioning the platform in a new (opposite) quadrant. Animals were tested in four trials according to the same procedure previously described. Besides the escape latency time, the time spent in both new and old quadrants were recorded. The time of escape latency and the path described to reach the platform (distance swam) were recorded for each trial.

Swimming strategies analysis

Swimming analysis was performed specifically in the long-term analysis time-point. Data collection and analysis of Morris Water Maze spatial reference trials were performed using a video-tracking system (Viewpoint, Champagne au mont d'or, France). Search strategies were defined as previously described [71]. Quantitative analysis and strategy classification was performed with data collected by the Viewpoint software, using an algorithm developed by us for systematic strategy attribution based on

pre-defined parameters. For strategies analysis, we defined two blocks of strategies: Block 1, comprising the "non-hippocampal-dependent strategies" (Thigmotaxis, Random Swim and Scanning) and Block 2, comprising the "hippocampal-dependent strategies" (Directed Search, Focal Search and Direct Swim); "chaining" was considered a transitional strategy and thus was not included in any of the blocks. Strategy blocks were defined as a sequence of at least three trials with the strategies from the same class. For block lengths, a maximum of two-trial interruptions were tolerated but not counted.

Forced swimming test (FST)

Depressive-like behavior was assessed through the forced swimming test. A FST trial was conducted 24 h after a 5-min pre-test session, by placing the rats in transparent cylinders filled with water (25 °C; 50 cm of depth) during 5 min. Trials were video-recorded and the immobility time, as well as the latency to immobility were measured using an automated video-tracking system (Viewpoint, Champagne au mont d'or, France). Learned-helplessness was considered as an increase in the immobility time.

Contextual fear conditioning (CFC)

CFC behavioral analyses were conducted specifically in the long-term analysis time-point. Contextual Fear Conditioning was conducted in white acrylic chambers with internal dimensions of 20 cm wide, 16 cm deep and 20.5 cm high (Med Associates), with an embedded light bulb mounted directly above the chamber to provide illumination. Each chamber contained a stainless-steel shock grid floor inside a clear acrylic cylinder, where animals were placed. Animals were subjected to two probes, a context probe and a cue (light) probe. The CFC procedure was conducted over 3 days (Fig. 4h), as follows: Day 1- Rats were placed in the conditioning white chamber (Context A) and received 3 pairings between a light (20 s) and a co-terminating shock (1 s, ≈0.7 mA). The interval between pairing was set as 180 s, and the first tone presentation commenced 180 s after the rat was placed into the chamber. Freezing was defined as the complete absence of motion, including motion of the vibrissae, for a minimum of 1 s. At the end of the three pairings, rats remained in the chamber for a further 30 s before being returned to their home cage. The chambers were cleaned with 10% ethanol solution between each trial. Day 2- For the context probe, animals were placed in the white chamber (Context A), where they were originally shocked, 24 h after the light-sock pairings. Freezing behavior was measured during 3 min. Two hours later, animals were put in a modified version of the chamber (Context B) that was covered with a black plastic sheet previously sprayed with vanilla oil, in order to alter both spatial and odor references. Additionally, the ventilation fan was turned off and the experimenter used different color gloves and lab coat. Again, freezing was measured during 3 min. Day 3- For the cued probe, animals were replaced in Context B, and at the end of variable period of 2 to 3 min a light was turned on (20 s). Freezing was measured in the subsequent minute.

Immunohistochemical analysis of cell proliferation and long-term survival

Animals ($n = 5$) were deeply anesthetized with sodium pentobarbital (60 mg/ml; 20%; Eutasil, Sanofi) and were transcardially perfused with cold 0.9% NaCl and 4% paraformaldehyde (PFA). Brains were removed, frozen and preserved at −20 °C. Coronal cryosections (20 µm) were firstly stained for BrdU (1:50; Dako, Glostrup, Denmark), to assess short-term hippocampal cell proliferation and survival both in the dorsal and ventral DG (dDG and vDG, respectively). Immunohistochemical analysis in the dorsal DG pole were performed in all brain sections comprised between 3.20 and 4.00 mm posterior to bregma; similarly, in the ventral pole, analysis was performed in all brain sections comprised between 5.40 and 6.20 mm posterior to bregma. For long-term cell survival analysis, sections from the "long-term" analysis group of animals (injected with BrdU 4 weeks before euthanasia) were co-stained with BrdU (1:50; Dako, Glostrup, Denmark), followed by sequential staining for NeuN (for mature neurons; 1:100; Chemicon, Temecula, CA, USA) or GFAP (for glia; 1:200; Dako). Finally, all sections were stained with 4′,6-diamidino-2-phenylindole (1 mg/ml). For each animal, BrdU-positive cells within the granule cell layer of the DG were analyzed after double staining with neuronal (NeuN) or glial (GFAP) markers and cell counts were performed by confocal microscopy (Olympus FluoViewTM FV1000, Hamburg, Germany).

Electrophysiological recordings

Electrophysiological recordings were obtained from anesthetized wt and GFAP-Tk rats (sevoflurane 3.5%; 650–700 mL/min). Rectal temperature was monitored and maintained at 37 °C using a homeothermic pad (Stoelting, Ireland). A surgical procedure was used to insert platinum/iridium concentric electrodes (Science Products, Germany) in the target positions as described previously [72]. following the Rat brain atlas (Paxinos): medial prefrontal cortex (mPFC, prelimbic region; coordinates, 1.94 mm anterior to bregma, 0.4 mm lateral to the midline, 2.54 mm below bregma); ventral hippocampus (vHIP; coordinates, 3.8 mm posterior to bregma, 3.3 mm lateral to the midline, 3.4 mm below bregma) and dorsal hippocampus (dHIP; coordinates, 3.2 mm posterior to bregma, 3.1 mm lateral to the midline, 2.5 mm below bregma). The subsequent electrophysiological characterization of the inter-regional link between vHIP and mPFC, and dHIP and mPFC was performed.

LFP signals obtained from the mPFC, vHIP, and dHIP were amplified, filtered (0.1–300 Hz, LP511 Grass Amplifier, Astro-Med, Germany), acquired (Micro 1401 mkII, CED, UK) and recorded running Signal Software (CED, UK). Local field activity was recorded at the sampling rate of 1000 Hz during 100 s.

At the end of the electrophysiological protocols, a biphasic 1 mA stimulus was delivered to electrodes to mark the place of recording. Rats were then deeply anesthetized with sodium pentobarbital, brains carefully removed, immersed in paraformaldehyde (PFA) 4% for 48 h and sectioned (50 µm) in the vibratome apparatus. Brain slices containing the mPFC, vHIP and dHIP were stained with Cresyl Violet for assessing the recording site. Animals with misplaced recording positions at least in one of the two regions under study were excluded from the analysis ($n = 5$ animals per group for each experimental condition).

Coherence between recorded regions was based on multi-taper Fourier analysis. Coherence was calculated by custom-written MATLAB scripts using the MATLAB toolbox Chronux (http://www.chronux.org). Coherence was calculated for each 1 s long segments and their mean was evaluated for all frequencies from 1 to 90 Hz. The power spectral density (PSD) of each assessed region was calculated through the 10× log of the multiplication between the complex Fourier transform of each 1 s long data segment and its complex conjugate. The PSD was evaluated for all frequencies from 1 to 90 Hz [72]. Both coherence and PSD measurements were assessed in the following frequencies: theta (4–12 Hz); beta (12–20 Hz); low gamma (20–40 Hz).

Data analysis and statistics

Statistical analyses were done using SPSS (SPSS, Chicago, IL, USA) and Graphpad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) softwares. The normal distribution was tested using the Kolmogorov–Smirnov test. Homogeneity of variances was assessed with Levene's test when different groups were compared and with Mauchly's Test of Sphericity when repeated measures were compared. ANOVA repeated measures was used to analyze spatial reference memory, working memory and LTP data. Unpaired *t*-test was used in the remaining analysis. Test statistics,

F-values and *p*-values are indicated along the text. Statistical significance was accepted for $p < 0.05$.

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AUTHOR CONTRIBUTIONS

JMM, BA, and TR maintained the GFAP-tk colony, induced the model, performed genotyping, and collected wellbeing measures. AMP and PP conducted all behavioral tests and immunohistochemical experiments, performed the analyses and interpreted the results. MMP and ELC assisted in the behavioral tests and analyzed the USV data. IC and VMS collected the electrophysiology data. IC, AJR, and JFO analyzed and interpreted the electrophysiology data. NDA and JSC processed all brains from electrophysiology experiments and for immunohistochemical analyses. AMP, PP, and LP designed the study, planned the experiments, and wrote the manuscript. AMP, PP, NS, JMB, and LP edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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