AP2γ controls adult hippocampal neurogenesis and modulates cognitive, but not anxiety or depressive-like behavior

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Hippocampal neurogenesis has been proposed to participate in a myriad of behavioral responses, both in basal states and in the context of neuropsychiatric disorders. Here, we identify activating protein 2γ (AP2γ, also known as Tcfap2c), originally described to regulate the generation of neurons in the developing cortex, as a modulator of adult hippocampal glutamatergic neurogenesis in mice. Specifically, AP2γ is present in a sub-population of hippocampal transient amplifying progenitors. There, it is found to act as a positive regulator of the cell fate determinants Tbr2 and NeuroD, promoting proliferation and differentiation of new glutamatergic granular neurons. Conditional ablation of AP2γ in the adult brain significantly reduced hippocampal neurogenesis and disrupted neural coherence between the ventral hippocampus and the medial prefrontal cortex. Furthermore, it resulted in the precipitation of multimodal cognitive deficits. This indicates that the sub-population of AP2γ-positive hippocampal progenitors may constitute an important cellular substrate for hippocampal-dependent cognitive functions. Concurrently, AP2γ deletion produced significant impairments in contextual memory and reversal learning. More so, in a water maze reference memory task a delay in the transition to cognitive strategies relying on hippocampal function integrity was observed. Interestingly, anxiety- and depressive-like behaviors were not significantly affected. Altogether, findings open new perspectives in understanding the role of specific sub-populations of newborn neurons in the (patho)physiology of neuropsychiatric disorders affecting hippocampal neuroplasticity and cognitive function in the adult brain.

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INTRODUCTION

In the adult central nervous system, specific brain niches retain the ability to generate new neurons throughout life.1 Among these, the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) is of particular interest. There, newly generated cells become mostly glutamatergic granular neurons,2-4 in the process recognized as neurogenesis. Adult hippocampal neurogenesis is a multistep and highly regulated process, originating from neural stem cells (NSCs) residing in the SGZ.1,5 Thereafter, the SGZ NSCs will divide to give rise to transient amplifying progenitors (TAPs), mitotically active cells, which will be responsible for the rapid expansion of the multipotent progenitor cells pool. Finally, the generated neuroblasts will undergo a short migration into the granule cell layer of the DG, differentiating into fully mature and integrated neurons in the pre-existing neural circuits. Importantly, survival of newborn cells depends on proper axonal and dendritic development. This confers cells the ability to receive GABAergic and, subsequently, glutamatergic synaptic input, both crucial for normal maturation and integration of newly generated cells.6

Several lines of evidence have shed light on the relevance of hippocampal neurogenesis for both structural and functional plasticity of the adult hippocampus. This process has behavioral repercussions in distinct cognitive and emotional domains, both in basal states and in neuropsychiatric disorders (such as schizophrenia and depressive disorders).7-11 More so, the transcriptional network involved in the regulation of neurogenesis, both in early developmental stages and during adulthood, has been the focus of recent studies.12-16 It is now established that during cortical development the regulation of glutamatergic neurogenesis is controlled by a set of transcription factors, including Pax6, Tbr2, NeuroD and Tbr1, with implications on proliferation, cell cycle kinetics, lineage and fate specification, axonal growth and cell adhesion processes.13,17,18 Interestingly, the transcriptional sequence of cell fate determinants (Pax6 → Tbr2 → NeuroD → Tbr1) is recapitulated during adult hippocampal neurogenesis and, with some variations, has a role in cell fate towards glutamatergic lineages in the subependymal zone.16,18-22

Activating protein 2γ (AP2γ, also known as Tcfap2c or Tgap2c) is a recently described transcription factor. It is part of the transcriptional network regulating glutamatergic neurogenesis during early developmental stages, directly regulating the basal progenitor fate determinants Math3 and Tbr2. In the developing cortex, deletion of AP2γ results in a specific reduction of upper layer neurons in the occipital cerebral cortex, whereas its overexpression potentiates region- and time-specific generation of cortical layers II/III.23 Yet, during adulthood, AP2γ has been classically linked to breast carcinogenesis, namely as a promoter of proliferation and impaired differentiation of tumor cells and as a positive regulator of the cell fate determinants Tbr2 and NeuroD, promoting proliferation and differentiation of new glutamatergic granular neurons. Conditional ablation of AP2γ in the adult brain significantly reduced hippocampal neurogenesis and disrupted neural coherence between the ventral hippocampus and the medial prefrontal cortex. Furthermore, it resulted in the precipitation of multimodal cognitive deficits. This indicates that the sub-population of AP2γ-positive hippocampal progenitors may constitute an important cellular substrate for hippocampal-dependent cognitive functions. Concurrently, AP2γ deletion produced significant impairments in contextual memory and reversal learning. More so, in a water maze reference memory task a delay in the transition to cognitive strategies relying on hippocampal function integrity was observed. Interestingly, anxiety- and depressive-like behaviors were not significantly affected. Altogether, findings open new perspectives in understanding the role of specific sub-populations of newborn neurons in the (patho)physiology of neuropsychiatric disorders affecting hippocampal neuroplasticity and cognitive function in the adult brain.

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a contributor to chemoresistance and radiation resistance of these cells.\textsuperscript{2,4}

Herein, in a mice model, we addressed the question of whether AP2\textgamma is an active transcriptional regulator of adult glutamatergic neurogenesis and if its function is relevant for different emotional and cognitive behavioral dimensions. The present study reveals an important role of AP2\textgamma in the regulation of glutamatergic neurogenesis in the adult hippocampal DG, with functional repercussions in the integrity of limbicocortical connections and in different cognitive modalities.

**MATERIALS AND METHODS**

A brief description of the Materials and methods is presented in this section. For a full description of all methods, please refer to the Supplementary Information.

**Animals**

AP2\textgamma\textsubscript{fl/+} (AP2\textgamma/\textgamma, Emx1-cre, Glast:CreERT2 (ref. 25) and Glast:CreERT2/Z/EG\textsuperscript{26} mice were maintained on a C57Bl/6J background (also used as wild type). For the initial in vivo AP2\textgamma deletion experiment, AP2\textgamma/\textgamma mice were crossed with Glast:CreERT2/Z/EG mice to generate AP2\textgamma/\textgamma/Glast:CreERT2/Z/EG mice. Tamoxifen (Sigma-Aldrich, St Louis, MO, USA; T-5648) was dissolved in corn oil (Sigma-Aldrich; C-8267) at 20 mg ml\textsuperscript{-1} and 1 mg was injected intraperitoneally two times a day for 5 consecutive days in 2-month-old male animals. Animals were killed 1 week after the end of tamoxifen administration. For in vivo AP2\textgamma overexpression experiments, 2-month-old male C57Bl/6J wild-type animals were stereotactically injected with 1 \mu\textgamma of either CAG-ires-GFP (ires-GFP) or CAG-ires-AP2\textgamma (AP2\textgamma-ires-GFP) retroviruses into the left and the right DG, and killed either 1 week or 1 month postinjections (\textit{n} = 5 per group for each experimental condition). For behavioral and electrophysiological studies, wild-type (Wt), AP2\textgamma/+/Glast:CreERT2 (AP2\textgamma/+/ko) and AP2\textgamma/+/Glast:CreERT2 (AP2\textgamma/–/– cKO) 2-month-old male mice were injected intraperitoneally with 1 mg tamoxifen two times a day for 5 consecutive days, with 7 days break followed by injections for 5 additional consecutive days. Animals were subjected to electrophysiological studies and behavioral testing 21 days after injections (\textit{n} = 10 per group).

All procedures were carried out in accordance with EU Directive 2010/63/EU and were approved by the Portuguese Government/Dire\cction Geral de Alimentação e Veterinária (DGAV) with the project reference 0420/000/000/2011 (DGAV 4542).

\textit{In situ} hybridization and immunohistochemical analysis

\textit{In situ} hybridization and immunostaining analysis were performed as described previously.\textsuperscript{25} Details on conditions and antibodies can be found in the Supplementary Information.

BrdU labeling

Wt mice used for cell type analyses with \textit{in situ} hybridization and immunofluorescence were given bromodeoxyuridine (BrdU) in drinking water (1 mg ml\textsuperscript{-1}; Sigma-Aldrich; B5002) for 2 weeks, and killed 8 weeks later. For the remaining deletion and overexpression experiments, mice were injected once with BrdU (100 mg kg\textsuperscript{-1}, intraperitoneally), 24 h before killing.

Primary DG cultures and \textit{in vitro} AP2\textgamma deletion

For primary DG cultures, six male mice (AP2\textgamma/\textgamma male mice, 2 months old) were used, as described previously.\textsuperscript{22} Cells were transduced with a retroviral vector iRES-GFP or CRE-iRES-GFP 2h after being plated.\textsuperscript{27} After 7 days in culture, cells were fixed with 4\% paraformaldehyde in PBS for 15 min. at room temperature and processed for antibody staining.

3D morphological analysis

To assess the 3D dendritic morphology of hippocampal DG granular neurons, we used the Golgi-Cox impregnation technique. Dendritic arborization and spine numbers/density were analyzed in the DG of Wt, AP2\textgamma/+/– cKO and AP2\textgamma/–/– cKO mice, as described previously\textsuperscript{7,9} (10–15 neurons for each animal; \textit{n} = 4 per group).

Electrophysiological studies

Local field potentials (LFPs) were recorded in the ventral hippocampus (vHIP) and in the prefrontal cortex (PFC); coherence measurements between simultaneously recorded LFPs in both regions were performed, as described previously.\textsuperscript{28} Power spectra densities (PSDs) were also measured in these two regions, as detailed in the Supplementary Information.

Behavioral analysis

Wt, AP2\textgamma/+/– cKO and AP2\textgamma/–/– cKO mice were tested in the forced swimming test (FST; to assess depressive-like behavior), in the open field and in the elevated plus maze tests (to assess anxiety-like behavior), as described previously.\textsuperscript{7} Furthermore, mice were tested in a contextual fear conditioning paradigm, as well as in different water maze tasks to characterize animals’ cognitive function, as detailed in the Supplementary Information.

Data analysis and statistics

Statistical analyses were performed using the SPSS software (Chicago, IL, USA). Animals were assigned to groups according to their genotypes. Sample sizes were determined by power analyses based on previously published studies. All presented data satisfied normal distribution in Kolmogorov–Smirnov testing. After confirmation of homogeneity of group variances between the groups, data were subjected to appropriate statistical tests. Analysis of variance (ANOVA) repeated measures was used to analyze performance on cognitive learning tasks. One-way ANOVA was used to evaluate the remaining behavioral and molecular results. F- and P-values derived from statistical analyses are properly indicated along the text. Differences between groups were determined by Bonferroni’s post \textit{hoc} multiple comparison test, and the corresponding \textit{P}-values are indicated in the figures. A\textit{t}-test was used to evaluate differences between two groups where appropriate. Statistical significance was accepted for \textit{P} < 0.05. No data points were excluded from the different analyses. Effect size, Cohen’s \textit{d} for \textit{t}-test and \eta\textsuperscript{2} for ANOVA were presented whenever statistical significance was reached. All results and corresponding statistical analyses are detailed in Supplementary Table 1.

**RESULTS**

AP2\textgamma is present in the adult hippocampal neurogenic niche

In light of the early description of the role of AP2\textgamma in the regulation of glutamatergic neurogenesis during developmental stages, we explored whether AP2\textgamma expression was present in the adult hippocampal DG, as this area represents an important source of glutamatergic neurons in the adult brain. Using \textit{in situ} hybridization to characterize regional gene expression distribution, we found AP2\textgamma-mRNA-positive cells in the adult DG (Figure 1a). Furthermore, using an 8-week BrdU label retaining protocol, we found colocalization of AP2\textgamma-mRNA signal with BrdU labeling, as well as with the transcription factor Tbr2, a regulator of glutamatergic neurogenesis in both developing and mature brain (Figure 1b). Subsequent immunofluorescent labeling of AP2\textgamma protein and cell count analysis revealed a high proportion of AP2\textgamma-positive cells in the SGZ to be also positive for the neuroblast marker doublecortin (DCX) (61.5 ± 2.7\%), whereas a subset of these cells was colabelled with Tbr2 (21.3 ± 4.1\%; Figures 1c and d), supporting lineage commitment of AP2\textgamma-positive cells to the glutamatergic neuronal lineage. Moreover, AP2\textgamma immunopositive cells were also positive for the cell cycle marker Ki-67 (Supplementary Figure 1) and BrdU (after an 8-week chase period) in the hippocampal DG (13.9 ± 3.5\%; Figures 1c and d), showing that a small portion of AP2\textgamma-positive cells are slow dividing progenitor cells. Importantly, we did not find colocalization between AP2\textgamma-positive cells and mature neuronal nuclei (NeuN)-positive neurons (Supplementary Figure 1).
AP2γ regulates adult hippocampal proliferation and neuronal differentiation, through reciprocal interactions with transcriptional regulators of glutamatergic neurogenesis

After identifying the presence of AP2γ in glutamatergic progenitors and neuroblasts of the adult DG, we assessed whether its ability to regulate neurogenesis during the prenatal cortical developmental window was preserved in the adult brain. To understand its role in neuronal fate specification, we used NSCs primary cultures, derived from the adult DG. We used a retroviral-based approach to infect cultured NSCs from mice containing AP2γ flanked by loxP sites (AP2γfl/fl mice) to delete AP2γ. Viral-mediated deletion of AP2γ produced a decrease in the generation of mixed clones (clones containing both neuronal Tuj1-positive and non-neuronal Tuj1-negative cells; \( t_{18} = 5.705, P < 0.001 \)), counterbalanced by a marked increase in the formation of non-neuronal Tuj1-negative clones (\( t_{18} = 7.173, P < 0.001 \)), supporting the role of AP2γ in commitment and differentiation into the neuronal lineage (Figures 2a and b). We did not observe a significant difference in the clone size of control and AP2γ-absent cells (Figure 2c).

To verify if the effects observed in vitro upon deletion of AP2γ were present in the adult brain, we used tamoxifen-inducible AP2γfl/fl/Glast::CreERT2/Z/EG mice (henceforth referred to as AP2γ\(^{-/-}\)) to promote the deletion of AP2γ, and evaluated the

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**Figure 1.** Activating protein 2γ (AP2γ) expression in the adult mouse hippocampal dentate gyrus (DG). (a) *In situ* hybridization (ISH) of AP2γ in the adult hippocampal DG. (b) The left panel shows the combination of ISH of AP2γ in the DG with immunolabelled bromodeoxyuridine (BrdU)-positive (in red) and Tbr2-positive cells (in green). (c and d) Immunohistochemical quantification of the percentage of AP2γ-positive cells colabelled with BrdU, Tbr2 or doublecortin (DCX) in the DG. Error bars represent s.e.m. Scale bars represent 100 μm (a) and 50 μm (b and c).
effects on hippocampal neurogenesis 1 week after induction (cells with AP2γ deletion become labeled as GFP-positive cells). In AP2γ−/− mice, we observed a significant decrease in the percentage of GFP/DCX-double-positive cells in the DG in comparison with Wt mice \((t_{18} = 4.239, P < 0.001)\) (Figure 2d). The decrease in neuroblasts was accompanied by an increase in GFP/GFAP-double-positive cells \((t_{18} = 4.171, P < 0.001; \text{Figure } 2d)\). This increase in GFAP-positive cells in the SGZ is likely to represent an increase in the GFAP-expressing progenitors pool, as a result of a defect in differentiation progression into glutamatergic neurons.

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We complemented these data with a forebrain AP2γ deletion experiment, and observed a decrease in DCX-positive neuroblasts in the DG (Supplementary Figure 2).

To gain further insight on the effects of AP2γ in the regulation of adult hippocampal neurogenesis, an AP2γ overexpression (AP2γox) experiment was conducted through intrahippocampal injections of a retrovirus carrying an AP2γ-IREs-GFP cassette in the DG. Hence, proliferative cells were stably infected by viral vectors, resulting in the overexpression of AP2γ and coexpression of GFP. Analysis performed 1 week after injection showed that a large proportion of GFP-positive cells corresponded to neuroblasts (GFP/DCX-double-positive cells; Figure 2e). Moreover, there was a reduction in the percentage of neuroblasts in AP2γox animals, 1 week after injection (t18 = 3.082; P = 0.003) (Figure 2e) that was accompanied by a significant increase in mature granular neurons (GFP/NeuN-double-positive cells; t18 = 4.945; P < 0.001) (Figure 2e) and a reduction in the GFAP-positive cell population (t18 = 2.828; P = 0.006) (Figure 2e). This result suggests the promotion of neurogenesis and an acceleration of the neuronal differentiation process after AP2γ overexpression. In animals killed 1 month after injection, most GFP-positive cells corresponded to mature (NeuN+)

Values were normalized to the pMXIG empty vector containing only GFP (four independent experiments). Student’s t-test, *P < 0.05, **P < 0.01 and ***P < 0.001. Error bars represent s.e.m. Scale bars represent 20 μm.

AP2γ ox animals present deficits in hippocampal proliferation (decrease in BrdU-positive cells), an effect that is more pronounced in homozygous mice (dorsal DG: F3,37 = 11.97, P < 0.001; post hoc: PC, P < 0.001; Figures 3b and c). Of note, AP2γ deficiency triggered a decrease of Pax6 and Tbr2 protein levels, but not Sox2 (an upstream regulator), in the both dorsal and ventral DG of adult mice (Figures 3b and c).

AP2γ cKO mice present deficits in hippocampal proliferation (decrease in BrdU-positive cells), an effect that is more pronounced in homozygous mice (dorsal DG: F3,37 = 11.97, P < 0.001; post hoc: PC, P < 0.001; Figures 3b and c). Moreover, AP2γ deficiency triggered a decrease of Pax6 and Tbr2 protein levels, but not Sox2 (an upstream regulator), in the both dorsal and ventral DG of adult mice (Figures 3b and c).

To explore whether AP2γ deficiency could affect other forms of structural plasticity within the adult DG, we analyzed the dendritic morphology of DG granular neurons, and spine densities and morphology (Figures 3g–i and Supplementary Figure 4). Of note, none of these parameters was affected by AP2γ deletion.

AP2γ deficiency induces cognitive deficits, but has no impact on anxiety- or depressive-like behavior.

Given the role of AP2γ in adult hippocampal neurogenesis, we tested AP2γ cKO mice in different behavioral paradigms to assess its impact in several emotional and cognitive domains. We used two behavioral tests to detect anxiety-like behavior, namely the open-field test and the elevated plus maze. AP2γ deletion was not sufficient to produce a statistically significant decrease in the total distance traveled in the center of the open-field arena (Figure 3j), or a decreased exploration time in open arms of the elevated plus maze (Figure 3k). Moreover, in the FST, a depressive-like behavior test, AP2γ cKO mice displayed similar immobility levels compared with WT animals (Figure 3l).

Next, we assessed the repercussions of AP2γ deletion for different cognitive domains. We tested animals in a contextual fear conditioning task, previously described to be sensitive to neurogenesis impairments.29 Animals were submitted to a context probe, aimed to test hippocampal-dependent memory, and a light-cued probe, aimed to assess the integrity of extrahippocampal memory circuits29 (Figure 3m). All groups presented similar average (DG) and normal freezing percentages after the conditioning trials (Figure 3m). In the context probe (context A), AP2γ−/− cKO presented a reduction in the percentage of freezing when exposed to a familiar context (F3,15 = 3.767, P = 0.047; post hoc: PC, P < 0.05; Figure 3m). Switching to a new environment (context B) promoted a decrease in freezing in all groups (Figure 3m). Of note, heterozygous deletion of AP2γ was not sufficient to produce impairments in contextual memory. In the light probe, all groups presented similar responses to the light cue (t1,4 = 0.7959, 0.7959).
Figure 3. Loss of activating protein 2γ (AP2γ) impairs adult hippocampal neurogenesis and cognitive function. (a) Two-month-old wild-type (Wt), AP2γ+/+/Glast-CreERT2 (AP2γ+/− cKO) and AP2γ+/−/Glast-CreERT2 (AP2γ−/− cKO) animals were injected with tamoxifen, tested 21 days after and subsequently killed. (b and c) Western blot analysis of AP2γ, Sox2, Pax6 and Tbr2 in adult hippocampal protein extracts from Wt, AP2γ+/− cKO and AP2γ−/− cKO mice; n = 5–6. (d) Dorsal hippocampal coronal section stained for bromodeoxyuridine (BrdU) (in green) and doublecortin (DCX) (in red). Double-stained BrdU and DCX are indicated by white arrows. (e and f) Cell counts of BrdU-positive cells and BrdU/DCX-double-positive cells in the hippocampal dentate gyrus (DG); n = 6. (g) Representative three-dimensional (3D) morphometric reconstruction of a DG granular neuron. (h and i) Dendritic length and spines density and morphology of hippocampal granular neurons; n = 10. (j and k) Anxiety-like behavior was tested both in the open-field test (j) and in the elevated plus maze (k). (l) The presence of depressive-like behavior was assessed in the forced swim test. (m) In addition, animals were tested in a contextual fear conditioning paradigm; percentage of freezing is presented after initial light-shock pairings (left panel), in the context probe (middle-right and -left panels) and in the cue probe (right panel); n = 10. One-way analysis of variance (ANOVA), *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001. Error bars represent s.e.m. Scale bars represent 50 μm. CFC, contextual fear conditioning; cKO, conditional knockout; EPM, elevated plus maze; FST, forced swim test; M, mushroom spines; OA, open arms; OF, open field; R, ramified spines; T, thin spines; Tk, thick spines.
Overall, contextual fear conditioning results showed that AP2γ−/− cKO display specific deficits in contextual hippocampal-associated memory, whereas preserving associative non-hippocampal-dependent memory.

We proceeded with the cognitive characterization of AP2γ cKO using different water maze test paradigms (Figure 4). In a reference memory task, which relies on the integrity of hippocampal function,30 Wt and AP2γ−/− cKO mice presented similar learning curves (Figure 4a). However, analysis of the strategies adopted to reach the escape platform31–33 showed that AP2γ−/− cKO mice delayed the switch from non-hippocampal-dependent strategies (‘Block 1’) to hippocampal-dependent strategies (‘Block 2’) (Figures 4b–h). In fact, most AP2γ−/− cKO animals initiate Block 2 strategies by test days 3 and 4, while presenting an increased mean duration of Block 1 compared with Wt mice (Block 1: \( t_{18} = 1.966; P = 0.032 \); Block 2: \( t_{18} = 2.690; P = 0.008 \); Figures 4e–h). Furthermore, in a working memory test paradigm, AP2γ−/− cKO and Wt mice presented similar performances along all the trials (Figure 4i). Regarding behavioral flexibility, AP2γ−/− cKO displayed increased time spent on the

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**Figure 4.** Cognitive strategies during water maze learning in AP2γ−/− conditional knockout (cKO) mice. (a–h) Spatial reference memory was evaluated as the average escape latency in each test day. A schematic representation and color code for each strategy (b) and the average prevalence of each strategy by trial number are shown both for wild-type (Wt) (c) and for AP2γ−/− cKO animals (d). The prevalence of each strategy-block along trials (Block 1: ‘non-hippocampal-dependent strategies’; Block 2: ‘hippocampal-dependent strategies’), the distribution of strategies-block boundaries and overall block length are shown for Wt (e) and AP2γ−/− cKO animals (f); graphical comparison of these parameters is shown in (g and h). (i and j) Furthermore, animals were tested in a working memory task (i) and in reversal learning task (j); \( n = 10 \); Student’s \( t \)-test, \( ***P < 0.001 \). Error bars represent s.e.m. AP2γ, activating protein 2γ.
In addition, and although still a matter of debate, altered hippocampal neurogenesis has been implicated in the precipitation of anxiety- and depressive-like behavior in rodent models of psychiatric diseases, as well as in the improving effects mediated by different classes of antidepressants, antipsychotic or antide-
mentia drugs.10,39,40 Herein, we investigated whether the neuro-
regulatory effects of AP2γ in the developing brain could be extended to the mature adult brain.

We believe the present study demonstrates for the first time the presence of AP2γ in the adult hippocampal DG, both in Tbr2-
positive glutamatergic progenitor cells and in neuroblasts. More-
over, results reveal that AP2γ is a positive regulator of adult hippocampal neurogenesis. Its overexpression promotes the generation of new neurons in this region, whereas its deletion results in a marked reduction of the neuroblast population, both in vitro and in vivo. Mechanistically, AP2γ acts as an effector of Sox2 and Pax6 in the promotion of Tbr2 expression in hippocampal progenitors. Indeed, Tbr2 (along with transcription factors such as NeuroD) is likely a major downstream effector of AP2γ regulatory pathway using AP2γ as an intermediate transcriptional regulator, in parallel with the direct regulation of Tbr2 by Pax6, in vivo.Mechanistically, AP2γ acts as an effector of Sox2 and Pax6 in the promotion of Tbr2 expression in hippocampal progenitor cells. In fact, we show that alterations in AP2γ expression produce a negative net effect in Tbr2 protein levels within the hippocampal DG (significant decrease). The results suggest that AP2γ regulates postnatal glutamatergic neurogenesis by mobilizing TAPs, rather than interfering with the NSC pool. Indeed, Tbr2 (along with transcription factors such as NeuroD) is likely a major downstream effector of AP2γ regulation. Tbr2 expression has been shown to be critical for TAPs’ pool expansion and to coordinate the progression to subsequent neuronal lineage differentiation stages in the adult hippocampus.22,41,42 Interestingly, the presence of an alternative regulatory pathway using AP2γ as an intermediate transcriptional regulator, in parallel with the direct regulation of Tbr2 by Pax6, suggests that AP2γ function may allow a fine-tuning of the neurogenic process. This may be either by rapidly expanding or by restricting the TAPs’ pool through the modulation of Tbr2.
expression. Accordingly, the reduction of progenitor cells observed after deletion of AP2γ possibly results from a failure in the progression to a postmitotic phase, where normal axonal growth and dendritic extension allow the proper synaptic input (shown to be critical for the successful survival and maturation of newborn cells). More so, AP2γ deletion in early embryonic corticogenesis was associated with a twofold increase in apoptosis of progenitor cells and their immediate progeny. Thus, it is plausible that the same developmental outcome is recapitulated in adult hippocampus and the observed reduction in TAPs is related with halted progression to subsequent maturation stages, culminating in cell death of glutamatergic progenitors.

We next explored how the transcriptional modulation of glutamatergic neurogenesis could impact on behavior. Interestingly, no significant changes were observed in emotional states, both depressive- or anxiety-like, in animals with reduced levels of AP2γ. Given that AP2γ is only present in a subset of newly formed neuroblasts, it is likely that the lack of AP2γ-positive neuroblast sub-population is not sufficient to elicit an evident phenotype. Moreover, AP2γ manipulation in the adult hippocampus did not influence normal dendritic morphology of postmitotic cells, another form of hippocampal structural plasticity critical for complex emotional behaviors. Altogether, results point for the need to characterize and modulate AP2γ-positive and -negative neuroblast populations in future studies. This will allow to pinpoint its specific participation in different behavioral outcomes, both in basal and in pathological context. Furthermore, it is plausible that by challenging the finely tuned hippocampal neurogenic process, AP2γ-positive newborn cells will evidence additional functional correlates. Accordingly, glutamatergic Tbr2-positive progenitors have been shown to be highly responsive to environmental enrichment or voluntary wheel running, which more than doubled Tbr2-positive TAPs, suggesting that in the advent of external stimuli these cells may have additional roles to those here reported. Additional insights on the full extent of the functional importance of AP2γ-positive progenitors may come from future studies analyzing the behavioral impacts of AP2γ overexpression in the adult hippocampus. More so, in studies, in which hippocampal neurogenesis has been experimentally bolstered, beneficial effects in learning, memory and pattern separation were reported. In the opposite perspective, in psychopathological contexts known to promote a potent antineurogenic insult, such as chronic stress exposure, the sub-population of AP2γ-positive progenitors is likely to become severely compromised. This reduction in AP2γ-positive cells, in articulation with other deleterious effects on neural plasticity promoted by chronic stress, may also contribute to a better characterization of the importance of these cells, not only in basal conditions but also in pathological scenarios, such as in depression.

Interestingly, AP2γ regulation of the TAPs’ population seems essential to the preservation of hippocampal-dependent cognitive tasks. Cognitive dimensions based on the interaction of the hippocampal formation and prefrontal cortical areas, such as spatial behavioral flexibility, were also impaired in AP2γ−/− KO animals. Strikingly, the electrophysiological studies revealed that AP2γ deficiency in the adult brain led to a significant decrease of coherence between the vHIP and the PFC, indicating a decrease in the ability of these regions to functionally interact. This included the θ and β frequencies, previously shown to be critically related with behavior outputs dependent on the corticolimbic networks. Such inter-regional electrophysiological impairments reflect how the lack of AP2γ-positive progenitors impact not only at the level of intrahippocampal circuitry but also modulate the function of cortical regions that cooperate with the hippocampus in the orchestration of complex cognitive behaviors. Moreover, the integrity of the vHIP-to-PFC link has been recently described to be important to the antidepressant action of drugs, such as ketamine, raising the possibility of AP2γ to have an important role in the preservation of this neuronal circuit.

Altogether, in this work we show that the lack of AP2γ in the adult mammalian brain impairs the regulation of hippocampal neurogenesis, leading to glutamatergic network malfunction impairments on neuronal activity and inter-regional communication. This dysregulation had significant implications for cognitive processes that may be relevant for the pathogenesis of psychiatric conditions. In light of the findings reported herein, future studies should explore whether AP2γ participates in the pathogenesis of these disorders characterized by hippocampal neurogenesis impairments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

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39 Kodama M, Fujikoa T, Duman RS. Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. *Biol Psychiatry* 2004; 56: 570–580.


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