Title: CBP is required for environmental enrichment-induced neurogenesis and cognitive enhancement

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Running title: CBP ans environment-induced neurogenesis

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SUMMARY

The epigenetic changes of the chromatin represent an attractive molecular substrate for adaptation to the environment. We examined here the role of CBP, a histone acetyltransferase involved in mental retardation, in the genesis and maintenance of long-lasting systemic and behavioral adaptations to environmental enrichment (EE). Morphological and behavioral analyses demonstrated that EE ameliorates deficits associated to CBP-deficiency. However, CBP-deficient mice also showed a strong defect in environment-induced neurogenesis and impaired EE-enhanced spatial navigation and pattern separation ability. These defects correlated with an attenuation of the transcriptional program induced in response to EE and with deficits in histone acetylation at the promoters of EE-regulated, neurogenesis-related genes. Additional experiments in CBP restricted and inducible knockout mice indicated that environment-induced adult neurogenesis is extrinsically regulated by CBP function in mature granule cells. Overall, our experiments demonstrate that the environment alters gene expression by impinging on activities involved in modifying the epigenome and identify CBP-dependent transcriptional neuroadaptation as an important mediator of EE-induced benefits, a finding with important implications for mental retardation therapeutics.

169 words

Introduction

Animals have developed a complex nervous system for adapting their conduct to the ever-changing environmental conditions. This flexibility at the behavioral level depends on the ability of neuronal circuits to evolve based on previous experiences through cellular mechanisms, such as functional and structural plasticity. These processes rely on the activation of specific and complex transcriptional programs. A novel idea that has come under discussion in recent years is that these gene programs are also subjected to activity-driven modulation through epigenetic modification of the chromatin of neural cells (Borrelli et al, 2008; Zocchi & Sassone-Corsi, 2010). Importantly, the malfunction of these processes can contribute to the molecular etiology of cognitive disorders (Graff & Mansuy, 2009). A good example of epigenetic disorder is the Rubinstein-Taybi syndrome (RSTS), a complex autosomal-dominant disease characterized by cognitive impairments and skeletal abnormalities (Rubinstein & Taybi, 1963; Wiley et al, 2003) associated to mutations in the gene encoding the CREB binding protein (CBP) (Petrij et al, 1995). CBP is a transcriptional co-activator with lysine acetyltransferase (KAT) activity and thereby it has the ability to leave epigenetic marks on the chromatin (Chan & La Thangue, 2001). The recent characterization of several mouse models for RSTS has demonstrated a direct role of the KAT activity of CBP in RSTS pathology and highlighted the importance of histone acetylation in neuronal plasticity and memory in the normal brain (Alarcon et al, 2004; Barrett et al, 2011; Bourtchouladze et al, 2003; Chen et al, 2010; Korzus et al, 2004; Valor et al, 2011; Viosca et al, 2010; Wood et al, 2006; Wood et al, 2005).

Environmental enrichment (EE) has been found to be beneficial in a number of cognitive disorders (Nithianantharajah & Hannan, 2006), including different forms

of mental retardation. Exposing laboratory rodents to continuous or repeated sessions of EE increase dendritic branching and spine number in hippocampal neurons, promotes neurogenesis and the integration of newborn neurons in functional circuits and improves learning and memory (Nithianantharajah & Hannan, 2006; van Praag et al, 2000). These events likely require the activation of complex gene networks, but the nature and sequence of the genetic program underlying these experience-driven changes in the structure and function of neuronal circuits remains elusive. Interestingly, EE has been shown to promote hippocampal histone acetylation (Fischer et al, 2007), a process that is impaired in RSTS mice (Alarcon et al, 2004).

We explore here the benefits of EE in a mouse model of RSTS mice. Our results reveal that EE promoted synaptic growth and alleviated some behavioral and cognitive deficits associated to RSTS. However, CBP-deficient mice showed a strong defect in environment-induced neurogenesis that correlated with attenuation of the transcriptional program associated with this process and with impaired EE-enhanced spatial navigation and pattern separation. Our results support an specific role for CBP in environment-induced neurogenesis and identify CBP-dependent transcription and histone acetylation as important mediators of environment-induced benefits.

Results

EE ameliorates some behavioral deficits in cbp$^{+/-}$ mice, but does not cause an improvement of spatial memory and discrimination

EE is known to trigger major structural and functional changes in the hippocampus (Nithianantharajah & Hannan, 2006). To examine the efficacy of behavioral therapy in RSTS, we compared cohorts of *cbp*$^{+/-}$ and control littermate mice housed either in

standard cages (SC) or in a large enriched environment (EE) in a number of paradigms.

We first examined whether EE promoted synaptic growth in this mouse models of RSTS by assessing dendritic spine density and morphology in CA1 pyramidal neurons of Thy1-EGFP-M/*cbp*$^{+/-}$ double mutants in different housing conditions. These animals exhibit an *in vivo* Golgi-like staining in the CA1 area in which few neurons are intensively labeled with EGFP expression (Feng et al, 2000). The examination of the number of dendritic spines in the *stratum radiatum* demonstrated that EE triggered similar structural changes in hippocampal pyramidal neurons of *cbp*$^{+/-}$ mice and control littermates (Figures 1A-B, spine density 2-way ANOVA: $F_{(1,24)\text{housing}} = 5.32$, $p = 0.03$). Neither EE nor CBP deficiency altered the morphology of dendritic spines and hippocampal dendritic organization (Supplemental Figure S1).

EE is also known to have important consequences in the animals' behavior (Nithianantharajah & Hannan, 2006). We observed that *cbp*$^{+/-}$ and control littermates behaved similarly in the open field and the elevated plus maze tasks (Supplemental Figures S2A-D) regardless of their housing conditions. EE, however, caused a remarkable improvement of the animals' performance in an accelerated RotaRod task in both genotypes (Figure 1C, latency to fall 2-way ANOVA repeated measures: $F_{(1,36)\text{genotype}} = 5.55$, $p = 0.02$; $F_{(1,36)\text{housing}} = 5.21$, $p = 0.03$), reversing previously described deficit of *cbp*$^{+/-}$ mice (Alarcon et al, 2004). The *cbp*$^{+/-}$-EE group stayed in the RotaRod as long as the WT-SC group, but did not reach the performance level of the WT-EE group. Importantly, the reversal of behavioral deficits was not restricted to locomotor impairments. *Cbp*$^{+/-}$ mice, like other CBP-deficient strains (Barco, 2007), are impaired in diverse memory tasks, including contextual fear conditioning

(Alarcon et al, 2004). We subjected cohorts of *cbp*$^{+/-}$ and control littermates housed either in SC or in EE to contextual fear conditioning and observed a similar effect of EE (Figure 1D, freezing time 2-way ANOVA: $F_{(1,35)\text{genotype}} = 13.52$, $p = 0.001$; $F_{(1,35)\text{housing}} = 10.72$, #: $p = 0.002$). The *cbp*$^{+/-}$-EE group showed similar memory as compared to the WT-SC group (*t*-tests; *cbp*$^{+/-}$ SC *vs* WT SC: $t_{(18)} = 3.76$, $p = 0.001$; *cbp*$^{+/-}$ EE *vs* WT SC: $t_{(17)} = 0.24$, $p = 0.81$), but lower than the WT-EE group (*t*-test; WT EE *vs* *cbp*$^{+/-}$ EE: $t_{(17)} = 2.14$, $p = 0.05$).

We also examined whether EE has a beneficial impact in the navigation skills of *cbp*$^{+/-}$ mice. In agreement with the reversal of motor deficits in the RotaRod task, the *cbp*$^{+/-}$-EE group increased their swimming speed in the Morris water maze (MWM) task (Supplemental Figure S2E), reversing the previously reported locomotor deficit (Alarcon et al, 2004). As previously reported (Alarcon et al, 2004), *cbp*$^{+/-}$ and control littermates maintained in standard cages performed equally well the hidden platform task in the Morris water maze ($F_{(1,16)\text{genotype}} = 2.44$, $p = 0.14$). As expected, EE improved the performance of wild type mice both during training ($F_{(1,17)\text{housing}} = 6.97$, $p = 0.02$, Figure 2A, upper panel) and in the two probe trials (Figure 2B, upper panel). In contrast, *cbp*$^{+/-}$ mice showed the same performance regardless of the housing condition ($F_{(1,14)\text{housing}} = 0.07$, $p = 0.79$, Figures 2A-B, lower panels). Interestingly, the large number of annulus crossings in the WT-EE group indicates that these animals show better spatial discrimination ability than the other three groups (Figure 2B).

To complete this comprehensive behavioral analysis, we assessed working memory and pattern separation ability in a water radial maze task (WRM) in which the mice were tested for the ability to select, from a choice of two arms, the arm harboring the escaping platform (Figure 2C). We performed four probe trials (T1 to

T4) and tested whether mice could differentiate between locations that were presented closely in space (LOW: T2 and T4) versus those that were more highly separated (HIGH: T1 and T3). C*bp*$^{+/-}$ mice were impaired in the spatial discrimination task (2-way repeated measures ANOVA: $F_{(1,30)\ \text{genotype}} = 5.55$, $p = 0.02$). In addition, only WT-EE mice performed equally well at low and high separations, whereas *cbp*$^{+/-}$ mice (regardless of the housing condition) and WT-SC mice could not discriminate between the choice and the target arms when presented in close spatial proximity (Figure 2C). These results indicate that EE enhanced pattern separation ability in wild type mice, an skill that is likely to be in higher demand when the animal lives in an enriched, changing environment.

Overall, our comprehensive behavioral analysis demonstrated that EE has a beneficial effect in various motor and cognitive abilities in *cbp*$^{+/-}$ mice and unveiled new deficits associated to impaired CBP function, namely reduced EE-enhanced spatial navigation and pattern separation ability.

CBP is specifically required for environment-induced neurogenesis

According to current theoretical models describing the role of the DG in the processing and storage of spatial information, newborn neurons in the SGZ are hypothesized to facilitate pattern separation and spatial memory resolution (Aimone et al, 2011; Deng et al, 2010; Sahay et al, 2011b). Therefore, our results in the MWM and WRM may suggest a specific defect in environment-induced neurogenesis. To assess this hypothesis, we examined adult neurogenesis in *cbp*$^{+/-}$ mice by BrdU uptake.

Adult neurogenesis is a complex and dynamic phenomenon that takes several weeks. Neural progenitors go through different stages before becoming mature

granule cells integrated in functional circuits (Kronenberg et al, 2003; Zhao et al, 2008). Five weeks after BrdU administration, most $BrdU^+$ cells (LRC, label-retaining cells) were double labeled with the marker for mature neurons NeuN (Figure 3A). In this condition, the quantification of LRC in the two neurogenic regions in the adult mouse brain, the subgranular zone (SGZ) and the subventricular zone (SVZ), demonstrated that basal adult neurogenesis is not affected in $cbp^{+/-}$ mice (Figure 3B-C and Supplemental Figure S3). We next examined EE-induced neurogenesis and found that EE increased more than 10-fold the number of newborn neurons in the SGZ of wild type mice, whereas $cbp^{+/-}$ mice only showed a modest increase (Figure 3B, $F_{(1,13)\text{housing}} = 40.05$, $p < 0.001$; $F_{(1,13)\text{genotype}} = 14.57$, $p < 0.01$; $F_{(1,13)\text{genotype x housing}} = 15.91$, $p < 0.01$). Our experiments therefore revealed a good correlation between EE-enhanced neurogenesis and spatial navigation and pattern separation abilities, supporting a specific role for newborn neurons in these skills.

To define more precisely the impairment associated to CBP deficiency, we examined the anatomy and cellular composition of the hippocampus of mutant mice and control littermates by magnetic resonance imaging (MRI, Supplemental Figure S4) and histological analyses (Supplemental Figure S5). C$bp^{+/-}$ mice had normal hippocampal structure, cell density and hippocampus/brain ratio despite of their slightly smaller body size, facial dysmorphia and skull abnormalities (Viosca et al, 2010). We also examined neuronal differentiation in the SGZ using different markers. The number of quiescent type-1 progenitors (radial $nestin^+$ cells) did not show significant difference between genotypes or housing conditions (Supplemental Figure S6A). In contrast, the examination of maturing neurons with doublecortin (dcx) immunolabeling revealed no difference between genotypes in the basal condition and a reduction in $cbp^{+/-}$ mice housed in an EE (Supplemental Figure S6B, $F_{(1,22)\text{housing}} =$

15.03, $p < 0.01$; $F_{(1,22)\text{genotype}} = 4.23$, $p = 0.05$; $F_{(1,22)\text{genotype x housing}} = 4.39$, $p < 0.05$). This result indicates that EE-induced neurogenesis is already impaired at early differentiation stages. In line with this finding, the determination of immature neurons by LRC count at earlier times after BrdU administration revealed no difference between genotypes in the basal condition (Supplemental Figure S6C).

We examined next whether CBP deficiency also interfered with activity-dependent regulation of neurogenesis in a paradigm unrelated with EE: the cellular response to a single injection of the pro-epileptic drug kainic acid (KA), a manipulation which is known to trigger long-term structural and functional changes in the hippocampus (Parent et al, 1997). As expected, KA-injected wild type mice displayed more BrdU/NeuN positive cells that those injected with vehicle, whereas there was no significant increase in neurogenesis in the case of $cbp^{+/-}$ mice (Figures 3C, $F_{(1,19)\text{treatment}} = 6.97$, $p < 0.05$; $F_{(1,19)\text{genotype}} = 5.65$, $p < 0.05$; $F_{(1,19)\text{genotype x treatment}} = 3.48$, $p = 0.08$).

CBP's paralog p300 has been also associated to RSTS, however the cognitive deficits associated to p300 deficiency both in humans and mice are more modest than for CBP (Viosca et al, 2010; Zimmermann et al, 2007). To evaluate the specificity of the role of CBP in environment-induced neurogenesis, we performed equivalent BrdU uptake experiments in $p300^{+/-}$ mice. This mutant strain showed normal adult neurogenesis in the SGZ both in standard conditions and after EE (Figure 4, $F_{(1,18)\text{housing}} = 31.17$, $p < 0.001$; $F_{(1,18)\text{genotype}} = 0.31$, $p = 0.59$).

Together, these results indicate that CBP plays a specific and important role in the adaptative response to tonic changes in the activity of hippocampal circuits triggered by experience.

Impaired transcriptional neuroadaptation and histone acetylation in the hippocampus of $cbp^{+/-}$ mice

To identify the genes downstream of CBP involved in these deficits, we determined the gene expression profile of CBP deficient mice and wild type littermates housed in SC or after two weeks of EE using microarrays. This analysis revealed that CBP deficiency had a very modest effect on basal gene expression, whereas EE triggered changes in more than 150 genes (Figure 5A).

None of the very few transcript clusters (TCs) significantly altered in the hippocampus of $cbp^{+/-}$ mice at the basal stage showed a FC larger than 1.2 (Supplemental Table 1). Interestingly, although the function of most of these low confidence candidate genes is poorly understood, some of them have been previously associated with mental retardation (e.g., *asl*, *spred2*, *srgap3*) and the development of the nervous system (e.g., *rtn4rl1*, *fjx1*, *crim1*) and may therefore contribute to RSTS neurological traits. As a validation of the microarray experiment, analysis of the individual probe sets comprised into CBP's TC confirmed a 50% reduction in the signal corresponding to exon 2, as it would be expected considering the gene targeting strategy used to generate these mice (Supplemental Figure 7A). Quantitative RT-PCR (qRT-PCR) assays using independent samples reproduced this result and confirmed that the reduction of CBP levels does not cause a compensatory upregulation of the paralog gene *Ep300* (Supplemental Figure 7B).

Regarding the transcriptional response to EE, our microarray analysis provided a comprehensive list of EE-induced genes in the mouse hippocampus (Supplemental Table S2). Interestingly, the *in silico* prediction tool PSCAN (Zambelli et al, 2009), which searches for TF consensus binding sequences in promoter regions, revealed a significant enrichment for CREB binding sites, among other CBP-

interacting transcription factors (Kasper et al, 2006), within the promoter region of these genes (Supplemental Table S3.). Gene Ontology (GO) analysis identified *Neurogenesis and neuron differentiation*, *Ion transport and homeostasis* and *Synaptic transmission* as the main biological functions affected by this condition (Figure 5E and Supplemental Figures S7C and Table S4).

The transcriptional program induced by EE was clearly attenuated in $cbp^{+/-}$ mice. Both gene upregulations and downregulations were affected (Figures 5A-D). Although all the GO functional groups listed above showed a significant interaction with genotype (Supplemental Figure S7D), *Neurogenesis and neuron differentiation* showed the most pronounced effect, manifested both in the number of entities affected and the level of statistical significance. The list of EE-regulated related to *Neurogenesis and neuron differentiation* (Supplemental Table S5) included 13 genes showing a significant genotype effect and 16 genes showing significant genotype x housing interaction. Figure 5F presents several examples of genes directly related to neurogenesis whose expression was enhanced in wild type mice housed in an enriched environment, but not in $cbp^{+/-}$ mice. Interestingly, PSCAN analysis revealed that the promoters of these genes were enriched in transcription factor binding site motifs recognized by some relevant CBP partners, such as NF-κβ, p53 and NRSF (Supplemental Table S6). Independent qRT-PCR assays for some of these genes, such as the pro-differentiative cytokine *lif*, the pro-neurogenic transcription factor *neurog1* and the microtubule-associate protein doublecortin (*dcx*), confirmed the microarray data (Figure 5G).

Given the cellular heterogeneity of the hippocampus, our gene profiling experiment cannot discriminate between transcriptional changes in specific cellular types and changes in the cellular composition of the tissue. Interestingly, the list of

neurogenesis-related genes showing a significant condition per genotype interaction (Supplemental Table S5) included both genes that likely play a cell autonomous effect (i.e., *nes, gli2*) and genes encoding for proteins that may have a paracrine function in neurogenesis (i.e. *lif)*, suggesting that both transcriptional changes in mature and newborn neurons can contribute to the altered gene profile. Importantly, in agreement with our observations regarding structural changes in CA1 neurons, the microarray data and independent qRT-PCR assays confirmed that CBP deficiency does not cause a general impairment in EE-regulated gene expression since we observed similar upregulation of a number of genes related to synaptogenesis, such as *bdnf*, *nptx2* and *vgf*, in both genotypes (Supplemental Figures 7E-F).

We next examined the acetylation state of mature neurons and neuroprogenitor cells in the DG of $cbp^{+/-}$ mice. Previous analyses in these mice had demonstrated a reduction in hippocampal histone H2B acetylation. This modification has been associated to active transcription (Karlic et al, 2010) and appears to be one of the main reaction catalyzed by the KAT activity of CBP *in vivo* (Alarcon et al, 2004; Valor et al, 2011). In agreement with this observation, we found that both, the proliferating cells in the SGZ (Figure 6A-B) and the surrounding mature granule cells (Figure 6C and Supplemental Figure S8A), have lower level of acetylated histone H2B (AcH2B) than the corresponding cells in wild type mice. We however did not observe any effect of long-term exposure to EE in the bulk acetylation level of different histones and specific lysine residues (Supplemental Figure S8B-C).

Next, we explored through chromatin immunoprecipitation (ChIP) assays whether the reduced bulk histone H2B acetylation affected the acetylation state of the promoters of some of the neurogenesis-related genes differentially induced by EE in $cbp^{+/-}$ mice and control littermates. Two-way ANOVA of ChIP data revealed

significant effects of both genotype and housing condition. Whereas CBP deficiency caused a significant reduction in the acetylation state of histone H2B, EE caused a significant increase. We also found that CBP deficiency prevented the increase of AcH2B induced by EE (Figure 7A). Interestingly, ChIP assays using antibodies against AcH3 showed that CBP deficiency also caused deficits in the acetylation of histone H3 at the promoters of the genes *dcx* and *nes* (Figure 7B) despite of the absence of significant deficits at bulk acetylation level (Supplemental Figure S8B-C). These local histone acetylation deficits were not restricted to the neurogenesis-related genes differentially induced in $cbp^{+/-}$ mice, NPY which is not affected at the transcription level by genotype also showed reduced levels of histone H2B acetylation at its promoters (Figure 7A-B).

Environment-induced neurogenesis is extrinsically regulated by CBP function in granule cells

To further clarify the role of CBP-dependent mechanisms in gene expression in environment-induced neurogenesis, we examined the consequences in adult neurogenesis of restricted ablation of CBP in mature neuron using CaMKIIa-creERT2/$CBP^{f/f}$ mice. These regulatable and restricted CBP knockout strain shows a complete ablation of CBP activity in mature granule neurons of the dentate gyrus few days after tamoxifen administration together with a dramatic reduction in the acetylation level of neuronal histones (Figure 8A and Supplemental Figure S9), whereas neuroprogenitor cells and immature neurons show normal levels of CBP and histone acetylation (Figures 8A-B). CaMKIIa-creERT2/$CBP^{f/f}$ and control littermates in SC and upon EE exposure received BrdU injections 12 weeks after tamoxifen administration. The quantification of the number of LRC in these four experimental

groups showed that the specific loss of CBP in mature granule cells is sufficient to cause impaired environment-induced neurogenesis in the SGZ (Figure 8C, $F_{(1,17)\text{housing}} = 62.77$, $p < 0.001$; $F_{(1,17)\text{genotype}} = 10.91$, $p < 0.01$; $F_{(1,17)\text{genotype x housing}} = 8.03$, $p < 0.05$). This result discards that the reported adult neurogenesis deficit were a consequence of developmental defects, and indicates that activity-dependent extrinsic regulation of hippocampal neurogenesis requires proper levels of CBP in mature granule neurons of the dentate gyrus.

Discussion

Our experiments in a mouse model for RSTS mental retardation demonstrate that EE ameliorates some of the deficits associated to RSTS and unveil new impairments caused by CBP-deficiency. $Cbp^{+/-}$ mice have a strong defect in environment-induced neurogenesis that correlates with the attenuated induction of EE-regulated genes, impaired EE-dependent histone acetylation at specific promoters, and with impaired pattern separation and spatial navigation abilities. Additional experiments with conditional knockout mice indicate that environment-induced neurogenesis is extrinsically regulated by CBP function in granule cells. Moreover, our rigorous microarray analysis contributes to elucidate the still poorly defined gene program induced in the hippocampus in response to environmental enrichment. This information will represent a useful resource for future studies since we identified dozens of new genes involved in the neuronal response to this condition. Although CBP deficiency had a minor impact in basal hippocampal gene expression, our screen also identified possible CBP downstream genes that may be relevant for RSTS pathology and should be further explored.

Reversal of behavioral deficits in cbp$^{+/-}$ mice: implications in RSTS therapeutics

Our interdisciplinary analyses demonstrate that long-term exposure to EE had a beneficial effect in various motor and cognitive abilities of *cbp*$^{+/-}$ mice and promoted synaptic growth and induced a number of plasticity-related genes in the hippocampus regardless of the genotype. Our data, therefore, suggest that behavioral therapy may be, as for other cognitive disorders (Nithianantharajah & Hannan, 2006), beneficial for RSTS children. However, EE also unveiled new deficits associated to CBP hemideficiency. The result of two independent water maze experiments, the classical hidden platform task and a radial maze task adapted to examine pattern separation ability, showed that *cbp*$^{+/-}$ mice, contrary to their control littermates, do not become more precise in the localization of the escape platform as result of the EE. There is a great deal of discussion about the role of hippocampal newborn neurons in learning and memory (Aimone et al, 2011; Deng et al, 2010; Ming & Song, 2011; Sahay et al, 2011b) and the extent to which increased-neurogenesis is relevant for cognitive improvement in hippocampal-dependent tasks (Jaholkowski et al, 2009; Meshi et al, 2006). Our behavioral experiments provide additional correlative evidence supporting the recently presented hypothesis that newborn neurons in the DG play a specific role in spatial memory resolution and pattern separation (Clelland et al, 2009; Creer et al, 2010; Sahay et al, 2011a). In addition, these results indicate that there are two components in the neurological deficits observed in RSTS mice, one of them susceptible of recovery through EE (e.g., locomotor and fear conditioning impairments) and another resistant to this condition and related to the production of newborn neurons in the SGZ. Overall, these findings suggest a role of defective neurogenesis in RSTS cognitive impairments and predict that the efficacy of environmental therapies might be more limited than in other mental impairment

disorders (Jirtle & Skinner, 2007). Our study might also have important implications for other neurological diseases since a similar specific defect in enrichment-mediated hippocampal neurogenesis has been reported for mice expressing Presenilin 1 (PS1) variants linked to early-onset familial Alzheimer's disease (FAD) (Choi et al, 2008) and for PS1 deficient mice (Feng et al, 2001). Since PS1 deficiency causes a reduction of the expression of CBP (Saura et al, 2004), our finding provides an attractive molecular explanation for FAD-linked defects in neurogenesis.

Role of CBP in environment-induced neurogenesis

Although CBP has been recently shown to regulate embryonic neural differentiation (Wang et al, 2010), our experiments indicate that a normal level of CBP is neglectable for steady-state neurogenesis in the adult hippocampus, but becomes again necessary for modulating the rate of neurogenesis in response to environmental challenges, such as EE and induced seizures, suggesting a general role for CBP in the adaptation of hippocampal circuits to external stimuli. CBP does so likely in coordination with other epigenetic factors like Gadd45b, a protein previously involved in DNA repair, whose loss also causes a deficit in environment-induced neurogenesis (Ma et al, 2009a). CBP plays a dual role in transcriptional regulation: it acts as transcriptional coactivator for a large number of transcription factors and as epigenetic enzyme with intrinsic KAT activity (Chan & La Thangue, 2001). It is likely that both functions will contribute to the neurogenesis and transcriptional defects observed in CBP deficient mice.

The most studied partner of CBP is CREB, an activity-regulated transcription factor that plays important roles in cognition (Benito & Barco, 2010) and adult neurogenesis (Merz et al, 2011). CREB regulates different phenomena during

neurogenesis, from proliferation and survival of neuroprogenitors to the maturation and integration of newborn neurons in neuronal circuits (Jagasia et al, 2009; Merz et al, 2011; Zhu et al, 2004). Other transcription factors involved in adult neurogenesis also use CBP as co-activator (Denis-Donini et al, 2008; Medrano & Scrable, 2005). These include NF-κβ and p53 that are, in addition, direct substrates of CBP's KAT activity (Ito et al, 2001; Nadiminty et al, 2006). Interestingly, we observed a strong and significant enrichment for the binding motifs of some of these transcription factors in the promoters of neurogenesis-related genes regulated by EE and showing a significant genotype effect or genotype x housing interaction.

Histone acetylation also plays an important role in neurogenesis (Lee & Lee, 2010). I*n vitro* experiments using HDAC inhibitors (HDACi), with few exceptions, have consistently shown that the treatment with HDACi reduces neural cell proliferation and promotes neuronal differentiation (Hao et al, 2004; Hsieh et al, 2004; Umka et al, 2010; Yu et al, 2009). However, the results of *in vivo* experiments are more difficult to interpret (Jessberger et al, 2007; Kim et al, 2009), probably because the reduction of proliferation by HDACi may result in a net reduction of neurogenesis in spite of the potential activity of these compounds to promote neuronal differentiation. Interestingly, the deficiency in HDAC2 results in specific and cell-autonomous defects in neural differentiation during adult neurogenesis (Jawerka et al. 2010). Therefore, the balance between histone acetylation/deacetylation seems to be critical for the correct activation and/or inactivation of neurogenic programs.

CBP and neuroadaptation to environmental changes

It has been proposed that epigenetic mechanisms, such as DNA methylation or histone modification, serve as key conduits for the extrinsic regulation of adult

neurogenesis by a wide variety of stimuli, including the environment and internal physiological states (Ma et al, 2009b; Ma et al, 2010). Since the recruitment of CBP to specific promoters is regulated by neuronal activity and depends on the sort of stimuli (Hardingham et al, 1999), this protein is in a privileged position to link neuronal circuit activity to epigenetic modification of the chromatin leading to persistent or permanent changes in neuronal circuits through changes in gene expression.

We and others have proposed that the reduction of CBP and the subsequent hypoacetylation of histones may interfere with the transcriptional response driven by activity, thus contributing to the cognitive deficits observed in mouse models for RSTS (Barco, 2007). Acetylation marks in the chromatin are considered a feature of active transcription (Kouzarides, 2007). In particular, the presence of acetylated H2B has been associated to highly transcribed genes and with the maintenance of transcriptional competence at specific *loci* (Karlic et al, 2010; Myers et al, 2003). Recent results indicate that this may be a consequence rather than a cause of the high transcriptional activity (Kasper et al, 2010; Valor et al, 2011). Histone acetylation, or at least some specific histone acetylation marks, may be associated to the ability to respond to certain stimuli rather than be an exact readout of the transcriptional activity of the loci. This would explain why the histone acetylation defects shown in Figures 6 and 7 do not have a direct correlate in basal hippocampal gene expression and basal adult neurogenesis. Given the role of CBP in the setting of epigenetic marks, it is maybe not surprising that the transcriptional consequences of its deficiency became more evident in the context of the establishment of a new long-term transcriptional stage, such as the response to EE. In agreement with this view, our microarray

experiment indicates that most of the transcriptional program induced by EE was affected by CBP deficiency.

The study of gene-environment interactions has experimented important progress in the last years. The epigenetic modification of the genome provides mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next (Jaenisch & Bird, 2003). Referring to neurons, the same epigenetic events can underlie the long-term maintenance, maybe for the whole life of the individual, of new gene activity states, providing a plausible link between experience and long-lasting alterations in gene expression in the brain (Fischer et al, 2007). In fact, recent studies have shown that some of the benefits of EE can be transmitted to the offspring, which necessarily involves the participation of epigenetic mechanisms (Arai et al, 2009). Our study demonstrates that the environment can alter gene expression and its functional outputs by impinging on activities involved in modifying the epigenome, highlighting the importance of neural histone acetylation in gene-environment interaction and adult neurogenesis (Hsieh & Eisch, 2010). It also identifies CBP-dependent transcription and histone acetylation as important mediators of environment-induced benefits.

Materials and methods

Maintenance, treatment and housing of transgenic mice

The generation of *cbp*$^{+/-}$ (Tanaka et al, 1997), *p300*$^{+/-}$ (Yao et al, 1998), CBP$^{f/f}$ (Zhang et al, 2004), CaMKIIa-creERT2 (Erdmann et al, 2007) and Thy1-EGFP (line M) (Feng et al, 2000) mice have been previously described. The experiments with *cbp*$^{+/-}$ mice were performed on a DBA and C57BL/6J mixed background, since these mutants are not viable in a pure C57BL/6J background (Alarcon et al, 2004). The genetic background of all other mice was C57BL/6J. Experiments were performed in 2-7 month old animals and in all cases the mice used as control were littermates of the mutant mice. For CBP ablation experiments, tamoxifen (T5648, Sigma-Aldrich) was administered to ~2 months old CaMKIIa-creERT2 /CBP$^{f/f}$ mice using a gastric probe for 5 consecutive days (total consumption=20 mg per animal); control animals were CaMKIIa-creERT2/CBP$^{f/f}$ treated with the same volume of the vehicle (corn oil C8267, Sigma-Aldrich) and non-cre recombinase expressing CBP$^{f/f}$ treated with tamoxifen. To observe nuclear translocation of cre recombinase in a control experiment, tamoxifen was administered once more the day before sacrifice. Mice were maintained according to animal care standards established by the European Union and all the protocols were approved by the Institutional Animal Care and Use Committee. The mice were kept on a 12h light/dark cycle and food and water were provided *ad libitum*. Standard housing consisted of 30x15x11 cm clear cages occupied by up to 5 mice. The environmental enrichment boxes were large white plexiglass boxes (>1m^2) and were occupied by a maximum of 20 mice. We used natural materials, plastic tubing, running wheels and toys to create an enriched environment whose configuration was modified every 48h shortly before the start of the dark cycle. The samples from EE animals used in the Western blot,

immunohistochemistry, qRT-PCR and microarray experiments were obtained at least 16 h after the last change to prevent any interference between the response to novelty and to EE. Intraperitoneal (i.p.) kainic acid injection (20 mg/kg in saline solution) caused overt seizures 5-10 min later, which lasted for approximately 2-3 h. before they spontaneously stopped. All mice displayed status epilepticus that started with jerking of the forelimbs, often followed by seesaws and involuntary falling. One death occurred among 12 animals both in the WT and in the *cbp*$^{+/-}$ group. For BrdU uptake experiments, the animals received i.p. injections of BrdU (100 mg/kg in saline solution) at the times indicated in the figure legends for the different experiments.

Antibodies

In this study the following primary antibodies were used: α-AcH2A, α-AcH2B, α-AcH3 and α-AcH4 (Sanchis-Segura et al, 2009); α-H2B (07-371), α-AcH2B-K5 (07-382), α-AcH2B-K12 (07-336), α-AcH2B-K15 (07-343), α-AcH2B-K20 (07-347), α-H3 (05-499), α-AcH3-K14 (06-911), α-AcH4-K8 (07-328), and α-NeuN (MAB377) from Millipore (Billerica, MA, USA); α-AcH2A-K5 (5276) from Cell Signaling (Beverly, MA, USA); α-H3 (ab1791), α-BrdU (ab6326), α-doublecortin (ab18723), α-nestin (ab11306), and α-AcH3-K14 (ab52946) from Abcam (Cambridge, UK); α-β-actin (F5441), α-GFAP (G9269), and α-MAP2 (M4403) from Sigma Aldrich Química S.A. (Madrid, Spain); α-GFP (A-11122) from Invitrogen (Carlsbad, CA, USA); α-CBP C1 from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA); α-Cre recombinase (Kellendonk et al, 1999). See Supplementary Information for secondary antibodies details.

Quantitative western-blotting

Western blot analyses were carried out as previously described (Sanchis-Segura et al, 2009). Equal amount of protein extracted from isolated hippocampi was loaded in each lane, and the intensity of the protein bands was measured using FUJIFILM LAS-100 equipment (Fujiphoto Film Co.) and quantified using Quantity One 4.6 software (Bio-Rad Inc.).

Immunohistochemistry and structural analyses

Mice were anesthetized with a ketamine/xylazine mixture immediately and perfused with paraformaldehyde (4% in 0.1 M phosphate buffer); brains were postfixed overnight. Immunostaining was performed on 50 μm free-floating sections. For BrdU staining, sections were incubated in HCl 2N for 30 min at 37ºC, rinsed in 0.1 M borate buffer, pH 8.5, and thoroughly washed in Tris-buffered saline, pH 7.4. For diaminobenzidine (DAB, Sigma) immunostaining, sections were pretreated with 0.6% H_2O_2 to block endogenous peroxidase reaction. For immunofluorescence, sections were counterstained with DAPI (Molecular Probes). For the structural analysis of dendritic spines, the transgenic line Thy1-EGFP (Feng et al, 2000) was crossed with *cbp*$^{+/-}$ strain to generate Thy1-EGFP/*cbp*$^{+/-}$ double mutants. Two-way ANOVA and two-tailed unpaired *t*-test were used to analyze histological data. The experimenters were blind to the genotypes and housing of the mice for all quantifications. See Supplementary Information for additional details on the analysis of dendritic spines and description of MRI methods.

Microarray analysis

Total RNA extracted from the hippocampi of three to four age, sex and genotype-matched mice were use to produce one pooled sample. We analyzed 16 samples (8

per genotype): 5 control (SC) and 3 enriched environment (EE). RNA samples were hybridized to GeneChip® Mouse Gene 1.0 ST Arrays according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). The microarray data was then analyzed using GeneSpring GX 11 (Agilent Technologies, Inc., Santa Clara, CA). Robust Multichip Average (RMA) algorithm was used for data normalization. Principal component analysis (PCA) revealed clustering of samples according to the batch of replicates; therefore normalization was conducted using the median of the corresponding control samples values as reference. Transcript clusters (TC) were then filtered on signal intensity by establishing a lower cutoff at the 20th percentile (20th to 100 percentile). Genotype and EE differentially regulated TC sets were obtained using 2-way ANOVA. TCs differentially regulated by "genotype" were also identified using unpaired-t-test since no TC passed the thresholds defined in the 2-way ANOVA analysis. In significance analysis, *p*-values were obtained by asymptotic computation and corrected for multiple-testing with Benjamini-Hochberg FDR method. Hierarchical clustering was also performed using GeneSpring software. Gene Ontology (GO) enrichment analysis for differentially regulated genes upon EE was performed using the web-based gene set analysis toolkit (WebGestalt) (Zhang et al, 2005). PSCAN Versión 1.1.1 was used for transcription factor binding site discovery (Zambelli et al, 2009). See Supplementary Information for additional detail. Microarray data are accessible through the Gene Expression Omnibus (GEO) database (GEO Series accession number GSExxxxx).

Quantitative RT-PCR and chromatin immunoprecipitation assays

Whole hippocampi were dissected and treated with RNAlater solution (Qiagen). DG subregions were microdissected from two 500-micron slices obtained from dorsal hippocampus with a tissue chopper (Stoelting). Total RNA was extracted using

RNeasy kit (Qiagen). Reverse transcription was performed using RevertAid First Strand cDNA synthesis kit (Fermentas). qRT-PCR was carried out using SYBR GreenER mix (Invitrogen, Carlsbad, CA) and primers specific for the genes of interest. ChIP experiments were performed according to the protocol in the Millipore ChIP kit (Millipore) with minor modification based on (Wells & Farnham, 2002). See Supplementary Information for addition details. Primer sequences used in ChIP and qRT-PCR assays are listed in Supplemental Table S7.

Behavior

For all behavioral tasks, we used adult female mutant and control littermates to prevent the fights observed between males housed in an enriched environment. The experimenters were blind to genotypes. The result of the PCR-based genotyping was provided as a factor for statistical analysis of the behavioral data once the battery of tasks was concluded. The open field, elevated plus maze RotaRod, Morris water maze and fear conditioning tasks were performed as previously described (Viosca et al, 2010). The training protocol in the MWM consisted of three trials per day with a 45 min inter trial interval. If the mice did not find the platform after 120s they were gently guided to it. Memory retention trials of 60s were performed at the beginning of day 5 (P1) and 24h after concluding the training on day 8 (P2). The number of annulus crossings was calculated considering an area double than the platform. For the pattern separation experiment, we adapted the protocol described into a water radial maze (Clelland et al, 2009). A six-arm water radial maze was positioned in the center of a pool (1.7 m of diameter) filled with opaque water. A platform of 10 cm diameter was placed 1 cm below the water level at the end of one selected arm. We positioned external cues in the walls to facilitate spatial navigation. The animals were trained per 3 consecutive days changing the platform position each day to ensure that

mice use a hippocampus dependent, allocentric strategy to solve the task; the platform position and the target arm were changed and balanced between trials. The animals were exposed each day to 14 training trials and 4 probe trials. In each trial, mice were gently positioned in the center of the maze facing to a closed arm and allowed to search for the platform for a maximum of 120 seconds. In the probe trials, the animals had to choose between two arms open (the target and the choice arms) at variable distance (HIGH: high spatial separation with one closed arm in between; or LOW: low spatial separation, in which the arms were contiguous). A trial was considered successful when the mouse entered into the target arm without making any error. See Supplementary Information for addition details.

Statistical methods

Precise description of the statistical methods used in each experiment is presented in the text. In the graphs, error bars represent s.e.m.

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Author contributions: JPLA and AB conceived and designed the study. AB wrote the manuscript. JPLA performed the gene expression analysis and most of the cellular and molecular biology work. AC and JV performed the behavioral experiments. AC and MG performed the dendritic spine analysis and some immunostainings with neuronal markers. LMV contributed to the analysis of CBP floxed mice. MJ assisted in the performance of immunostainings. SC performed de MRI analysis.

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FIGURE LEGENDS

Figure 1. EE-mediated structural, behavioral and cognitive benefits. **A.** Representative confocal images showing spines protruding from dendritic segments of hippocampal CA1 pyramidal neurons from *cbp*$^{+/-}$ mice and control littermates in SC or EE conditions. Scale bar: 5 μm. **B.** Mice exposed to EE, independently of the genotype, show increased density of dendritic spines compared to animals kept in SC. Two-way ANOVA, #: significant housing effect. n = 6-8 mice per group. These EE-dependent changes did not affect the morphology of dendritic spines (Supplemental Figures S1A-C) or the general cytoarchitecture of the hippocampus (Supplemental Figures S1D-E). **C.** *Cbp*$^{+/-}$ mice show deficits in RotaRod performance and the exposure to EE causes a recovery in mutant mice and an improvement of the performance of WT mice. Two-way ANOVA, #: significant housing effect, §: significant genotype effect. n = 10 mice per group. **D.** *Cbp*$^{+/-}$ mice show an impairment in contextual fear conditioning. EE rescues this deficit and improves the contextual memory of WT mice. Two-way ANOVA, #: significant housing effect, §: significant genotype effect. *t*-tests compared to WT SC group: *: $p < 0.05$; **: $p < 0.005$. n = 9-10 mice per group.

Figure 2. C*bp*$^{+/-}$ mice show impaired EE-enhanced spatial navigation and pattern recognition ability. A. The 2-way ANOVA analysis of path lengths in the water maze task revealed a significant housing effect ($F_{(1,31)\text{housing}} = 4.81$, $p = 0.04$), no genotype effect ($F_{(1,31)\text{genotype}} = 0.37$, $p = 0.55$), and indicated a possible genotype x housing interaction ($F_{(1,31)\text{genotype x housing}} = 3.53$, $p = 0.07$). More precisely, EE improved the performance in WT mice (upper panel, 2-way ANOVA, #: significant housing effect), but not in *cbp*$^{+/-}$ mice (lower panel, NS: non significant). n = 8-10 mice per group. **B.** EE housed wt mice showed more annulus crossings in the first and

the second probe trials (upper panels: P1, $p = 0.06$ no significant difference; P2, *: $p = 0.03$), whereas *cbp*$^{+/-}$ mice did not exhibit any housing effect (lower panels: P1, $p = 0.90$; P2, $p = 0.70$). **C.** Upper panels: Schematic representation of the water radial maze tests used to measure pattern separation. Mice were tested for their pattern separation ability by comparing their performance in two types of tests: low separation tests (LOW) in which the target arm (T, where the platform is located) and the choice arm (C) were contiguous, and high separation tests (HIGH) in which the target and the choice arms were separated by a closed arm. Graph: In the third day of training, the mice were subjected to two symmetrical low and high separation tasks and the average performance was calculated. WT mice housed in an enriched environment (WT EE) performed well both kind of tests (% of correct HIGH: $t_{(7)}= 2.65$, $p = 0.03$; % of correct LOW: $t_{(7)}$:3.42, $p = 0.01$), whereas WT housed in standard cages (WT SC) were only successful in the high separation tests (% of correct HIGH: WT SC, $t_{(9)}= 3.00$, $p = 0.02$). In contrast, *cbp*$^{+/-}$ mutants failed in both the HIGH and the LOW tests. *: $p < 0.05$ *t*-tests *vs* 50 (chance). n = 8-10 mice per group.

Figure 3. Impaired induced-neurogenesis in the SGZ of *cbp*$^{+/-}$ mice. A. Animals received two daily injections of BrdU (100 mg/kg) for 5 consecutive days starting at day 11 of EE and were perfused (P) 35 days later. The right image shows cells in the SGZ immunolabeled with antibodies against BrdU (red) and NeuN (green) and nuclei counterstained with DAPI (blue). Five weeks after the last administration of BrdU, the vast majority of surviving cells were NeuN$^+$. Scale bar: 2 μm. **B.** CBP deficient mice show a severe impairment in EE-induced neurogenesis in the SGZ. The right panels show representative images of BrdU (brown nuclei) immunostaining showing newborn neurons in the SGZ of WT and *cbp*$^{+/-}$ mice in SC and EE. Two-way

ANOVA, #: significant housing effect, §: significant genotype effect, &: significant genotype x housing interaction. n = 3-4 mice per group. Scale bars: 100 µm. **C.** KA-induced neurogenesis is also impaired in *cbp*$^{+/-}$ mice. Six days after a single administration of kainic acid (20 mg/kg) (KA) or vehicle (Veh), WT and *cbp*$^{+/-}$ mice received 2 daily BrdU injections for 5 consecutive days. Five weeks later, the mice were perfused (P) and adult newborn cells were stained for BrdU. The right panels show representative images of LRC immunolabeling in the SGZ of WT and *cbp*$^{+/-}$ mice treated with KA or vehicle. Two-way ANOVA, #: significant housing effect, §: significant genotype effect. n = 4-5 per group. Scale bars: 100 µm.

Figure 4. Environment-induced neurogenesis is not altered in *p300*$^{+/-}$ mice. p300 deficient mice show normal basal and EE-induced neurogenesis in the SGZ. The right panels present representative images of BrdU (brown nuclei) immunostainings. Two-way ANOVA, #: significant housing effect. n = 3-6 per group. Scale bar: 100 µm.

Figure 5. Impaired neuroadaptative transcriptional response to environmental enrichment in the hippocampus of *cbp*$^{+/-}$ mice. A. The hierarchical cluster of the 159 TCs differentially regulated in response to EE (corrected p value < 0.05, FC > 1.3) reveals an attenuated transcriptional response in *cbp*$^{+/-}$ mice. **B.** Number of TCs upregulated (white) and downregulated (black) in response to EE in WT and *cbp*$^{+/-}$ mice (referred to the respective SC groups) with FC > 1.3. **C.** Venn diagram showing the number of EE-regulated TCs. **D.** Scatter plot comparing, in WT and *cbp*$^{+/-}$ mice, the FC of the 84 TCs differentially upregulated in response to EE. The dotted line indicates the threshold for FC. Most dots are located in the upper left quadrant indicating that the changes are larger in WT animals. $r_{(82)} = 0.31$, $p < 0.05$. **E.** Pie diagram showing the number of unique entities associated to a GO term in each of the major functional categories identified in the analysis of gene sets differentially

expressed in EE mice. **F.** Bar graph showing the expression level of specific neurogenesis-related genes whose induction by EE is impaired in *cbp*$^{+/-}$ mice (expression values extracted from microarray data). Two-way ANOVA, §: significant genotype effect, &: significant genotype x housing interaction (non corrected *p* values). All these genes show a significant housing effect. Some interesting genes showing borderline significance are also presented. **G.** qRT-PCR validation of EE-mediated hippocampal induction for the neurogenesis-related genes *lif*, *neurog1* and *dcx*. Two-way ANOVA, #: significant housing effect, §: significant genotype effect, &: significant genotype x housing interaction. n = 3 per group.

Figure 6. Reduced histone acetylation in the dentate gyrus of *cbp*$^{+/-}$ mice. A. Representative confocal images of the DG labeled with BrdU (red) and acetylated histone H2B (green). Scale bar: 25 μm. **B.** Quantification of fluorescence intensity in individual BrdU^{+} cells demonstrates that the acetylation of histone H2B is reduced in the proliferating neuroprogenitors of *cbp*$^{+/-}$ mice. *: $p < 0.05$ (unpaired two-tailed *t* test), n = 12-16 cells per group. **C.** Quantification of fluorescence intensity in individual granule cells (BrdU^{-} cells adjacent to the BrdU^{+} cells shown in Panel B) demonstrates a general reduction of histone H2B acetylation in the DG of *cbp*$^{+/-}$ mice. *: $p < 0.05$ (unpaired two-tailed *t* test), n = 46-50 cells per group.

Figure 7. Reduced histone acetylation at the promoters of neurogenesis-related genes. A. ChIP assays using an antibody against AcH2B. **B.** ChIP assays using an antibody against AcH3. Two-way ANOVA, #: significant housing effect, §: significant genotype effect. n = 3 mice per sample, 3 samples per condition.

Figure 8. The environment-induced neurogenesis defect is not developmental and depends on intact levels of CBP in mature granule cells. A. In forebrain-restricted inducible CBP knockout mice, tamoxifen injection causes the elimination of

CBP immunoreactivity (red) in granule cells and severe hypoacetylation (green). The few remaining CBP^+ cells in the inner blade of the DG show normal levels of AcH2B (arrow heads) and are $NeuN^-$ (see Supplemental Figure S9), suggesting that only progenitors and newborn neurons still express CBP. Nuclei were stained with DAPI (blue). Scale bar: 50 μm. **B.** Immunohistochemistry for CBP (red) and DCX (green) in coronal sections of CaMKIIa-creERT2/$CBP^{f/f}$ and WT/$CBP^{f/f}$ mice treated with tamoxifen demonstrate that most of the remaining CBP^+ nuclei in the SGZ of CaMKIIa-creERT2/$CBP^{f/f}$ mice belong to Dcx^+ cells (solid arrowheads). The empty arrowheads denote cells in the SGZ of the DG that were CBP^+ and Dcx^-. Nuclei were stained with DAPI (blue). Right, magnification of the dotted squares in the left images show CBP^+ and DCX^+ cells in the SGZ of the DG. Scale bar: 15 μm. **C.** Tamoxifen was administered to 2 month-old mice and twelve weeks later, mice were housed in EE or maintained in SC. Animals received two daily injections of BrdU (100 mg/kg) for 5 consecutive days starting at day 11 of EE. Five weeks after the last administration of BrdU, the animals were perfused (P) for immunohistochemistry. CaMKIIa-creERT2/$CBP^{f/f}$ mice show a severe impairment in EE-induced neurogenesis in the SGZ. The right panels show representative images of BrdU (brown nuclei) immunostaining showing newborn neurons in the SGZ of CaMKIIa-creERT2/$CBP^{f/f}$ mice and control littermates housed either in SC or EE. Two-way ANOVA, #: significant housing effect, §: significant genotype effect, &: significant genotype x housing interaction. n = 4-5 per group. Scale bar: 100 μm.

Figure 1

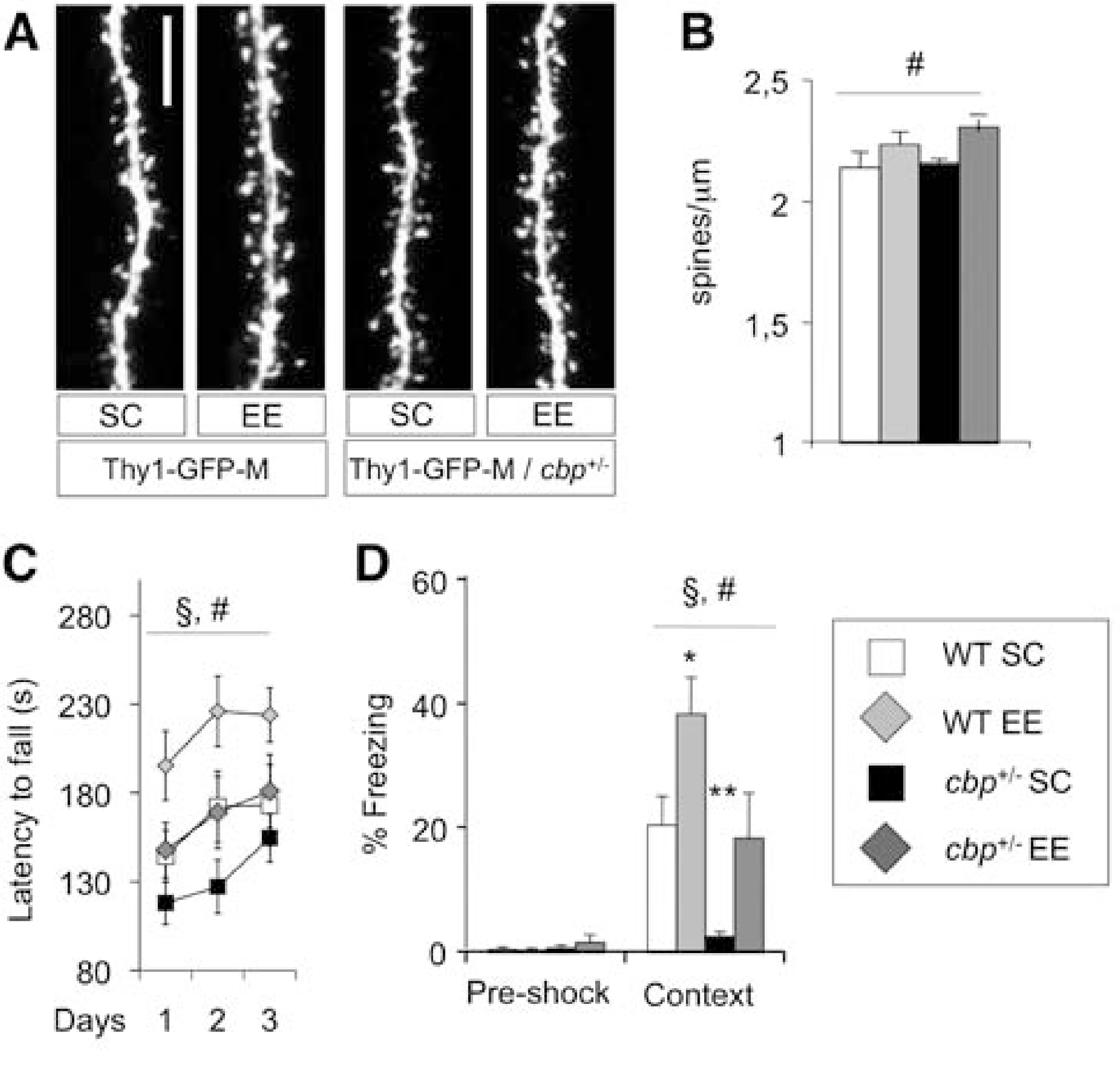

A
SC
EE
SC
EE
Thy1-GFP-M
Thy1-GFP-M / cbp+/-
B
#
2,5
2
1,5
1
spines/µm
C
§, #
280
230
180
130
80
Latency to fall (s)
Days 1 2 3
D
§, #
*
**
60
40
20
0
% Freezing
Pre-shock
Context
WT SC
WT EE
cbp+/- SC
cbp+/- EE

Figure 2

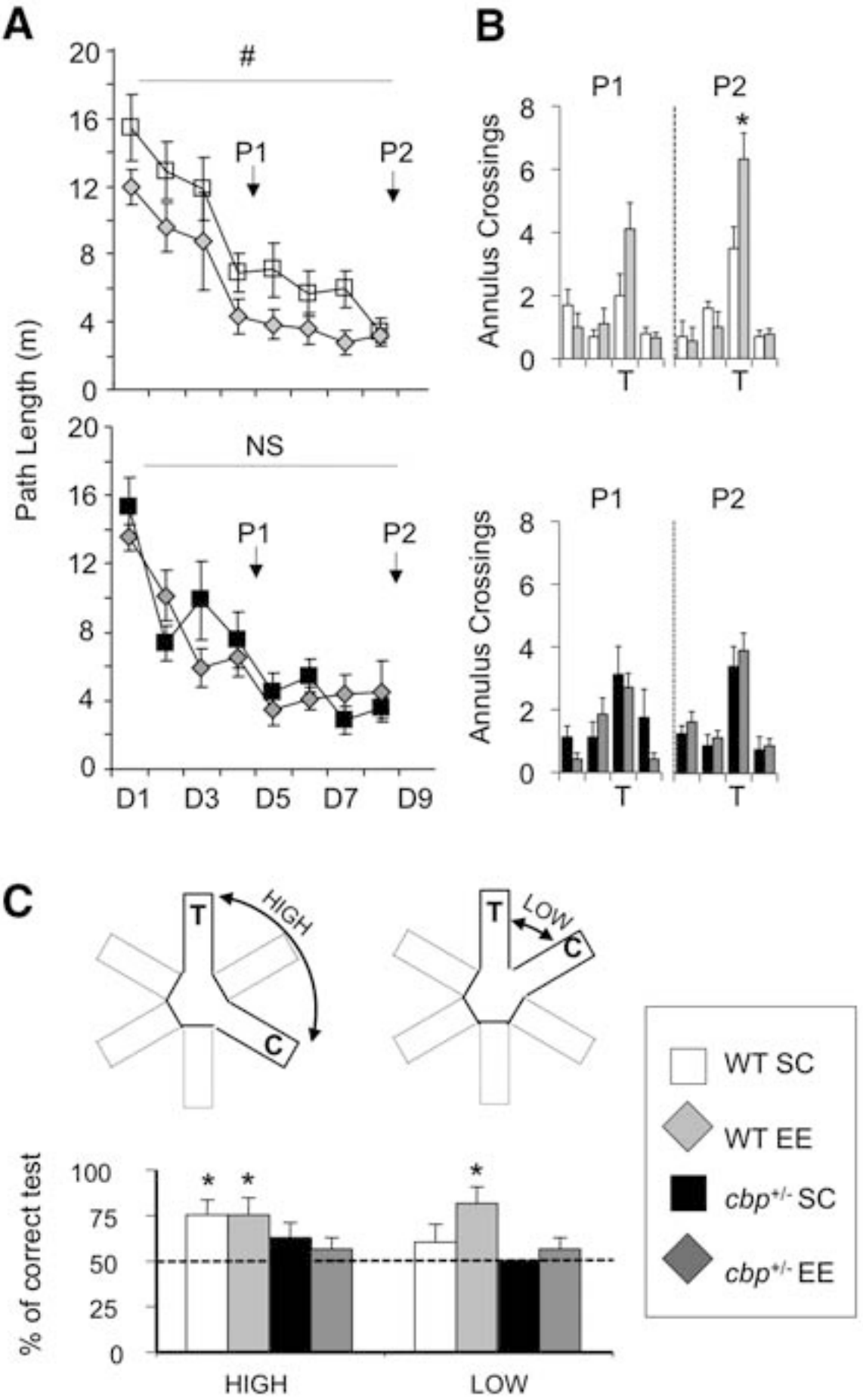

A
B
C
#
NS
P1
P2
Path Length (m)
Annulus Crossings
T
D1
D3
D5
D7
D9
HIGH
LOW
% of correct test
WT SC
WT EE
cbp+/- SC
cbp+/- EE

Figure 3

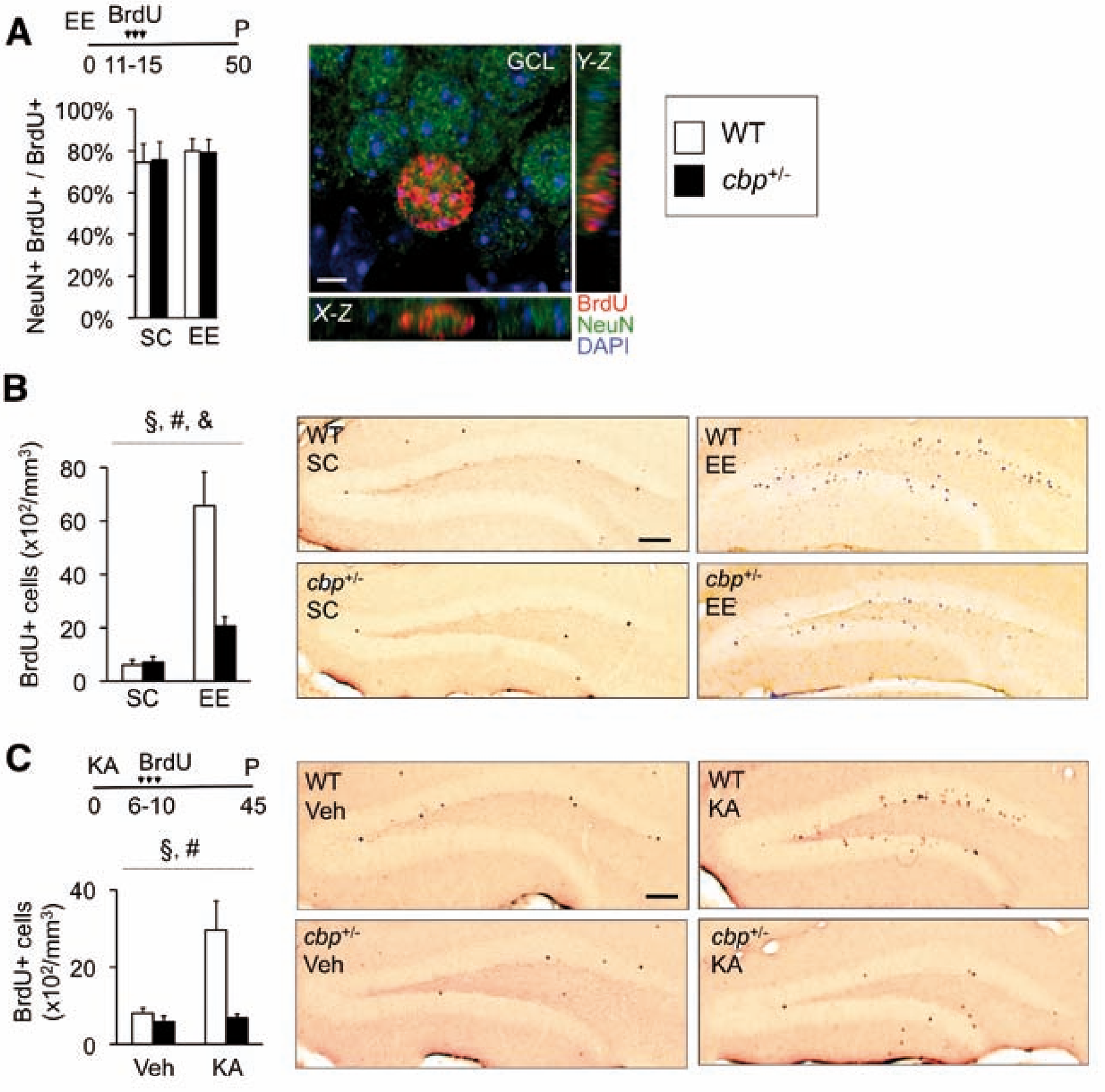
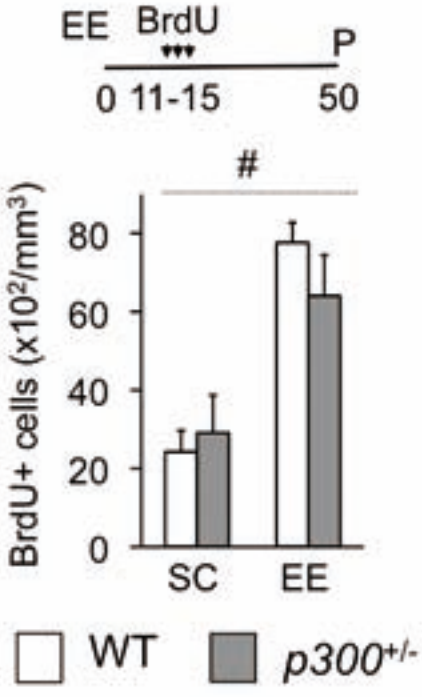
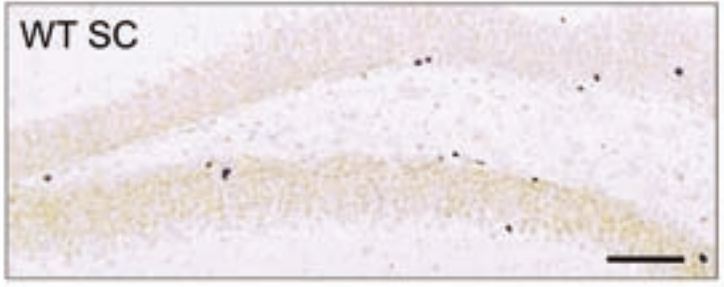
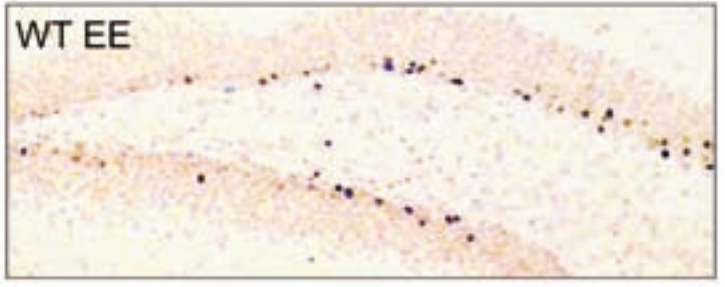

A
EE BrdU P
***
0 11-15 50
NeuN+ BrdU+ / BrdU+
100%
80%
60%
40%
20%
0%
SC EE
GCL
Y-Z
X-Z
BrdU
NeuN
DAPI
WT
cbp+/-
B
§, #, &
BrdU+ cells (x10²/mm³)
80
60
40
20
0
SC EE
WT SC
WT EE
cbp+/- SC
cbp+/- EE
C
KA BrdU P
***
0 6-10 45
§, #
BrdU+ cells (x10²/mm³)
40
20
0
Veh KA
WT Veh
WT KA
cbp+/- Veh
cbp+/- KA

Figure 4


EE
BrdU
P
0
11-15
50
#
BrdU+ cells (x10²/mm³)
80
60
40
20
0
SC
EE
WT
p300+/-


WT SC


WT EE


p300+/- SC

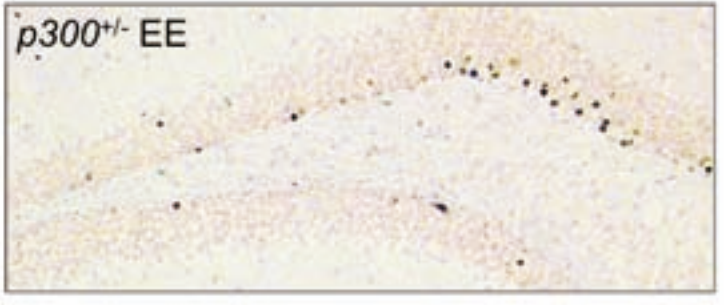

p300+/- EE

Figure 5

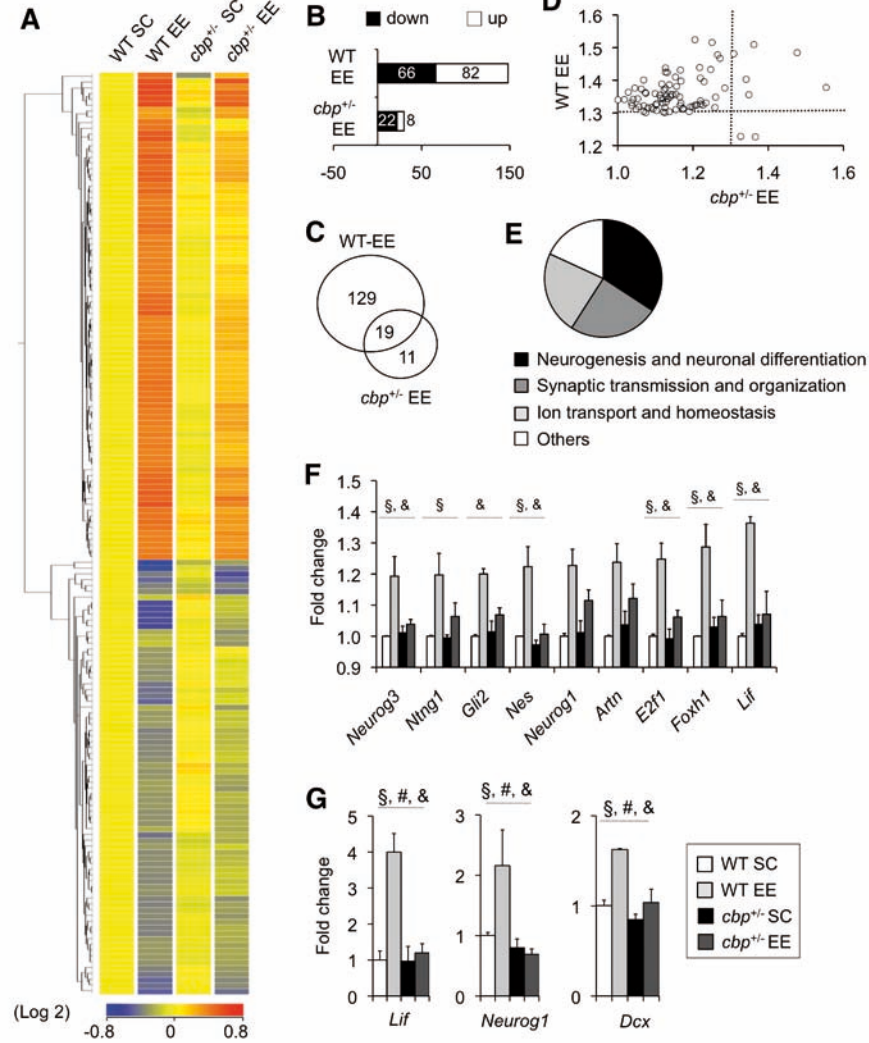

A
WT SC
WT EE
cbp+/- SC
cbp+/- EE
(Log 2)
-0.8
0
0.8
B
down
up
WT EE
66
82
cbp+/- EE
22
8
-50
50
150
C
WT-EE
129
19
11
cbp+/- EE
D
WT EE
cbp+/- EE
1.0
1.2
1.4
1.6
1.3
1.5
E
Neurogenesis and neuronal differentiation
Synaptic transmission and organization
Ion transport and homeostasis
Others
F
Fold change
§, &
§
&
§, &
§, &
§, &
§, &
Neurog3
Ntng1
Gli2
Nes
Neurog1
Artn
E2f1
Foxh1
Lif
G
Fold change
§, #, &
§, #, &
§, #, &
Lif
Neurog1
Dcx
WT SC
WT EE
cbp+/- SC
cbp+/- EE

Figure 6

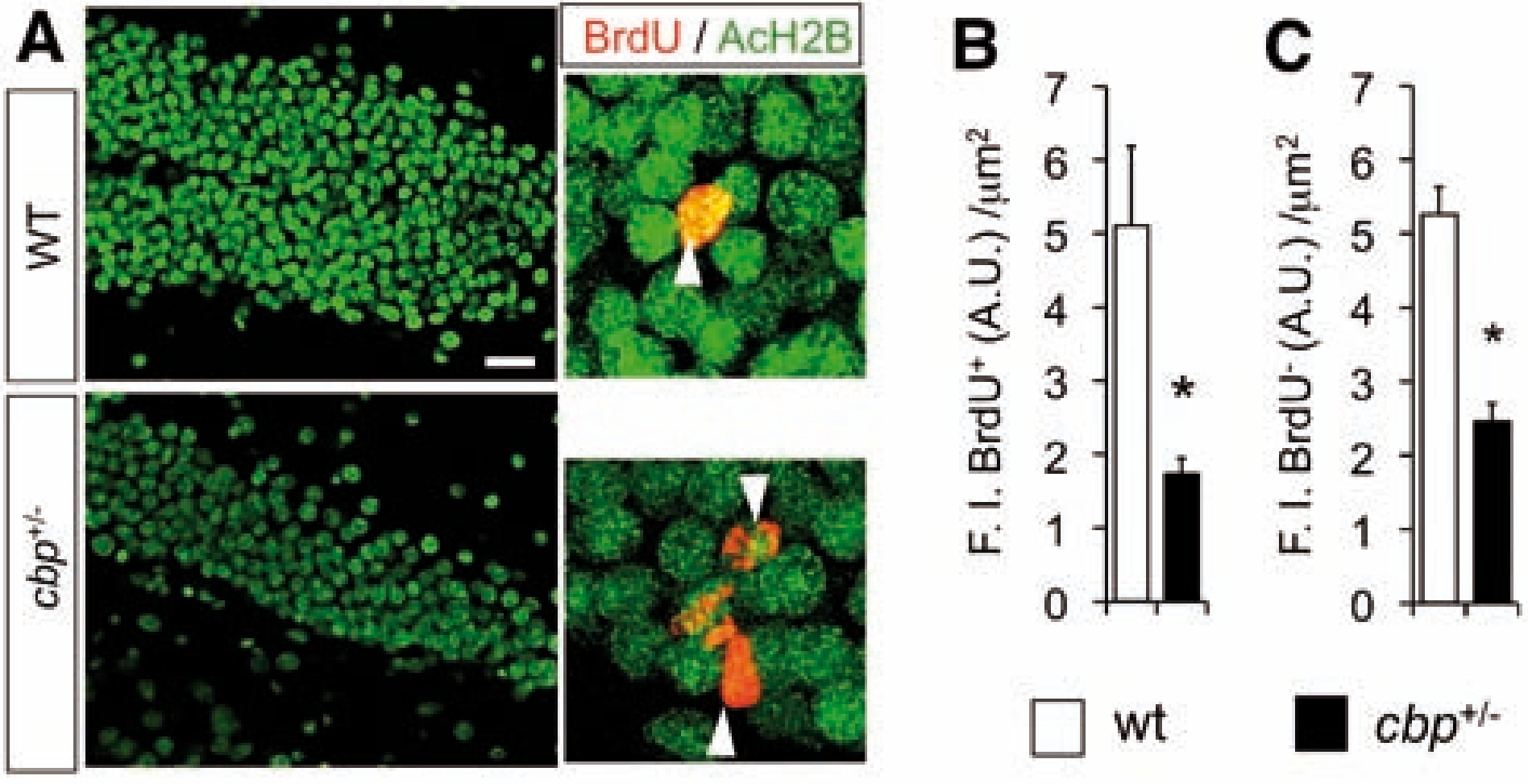

A
BrdU / AcH2B
WT
cbp+/-
B
F. I. BrdU+ (A.U.) /µm2
0
1
2
3
4
5
6
7
*
C
F. I. BrdU- (A.U.) /µm2
0
1
2
3
4
5
6
7
*
wt
cbp+/-

Figure 7

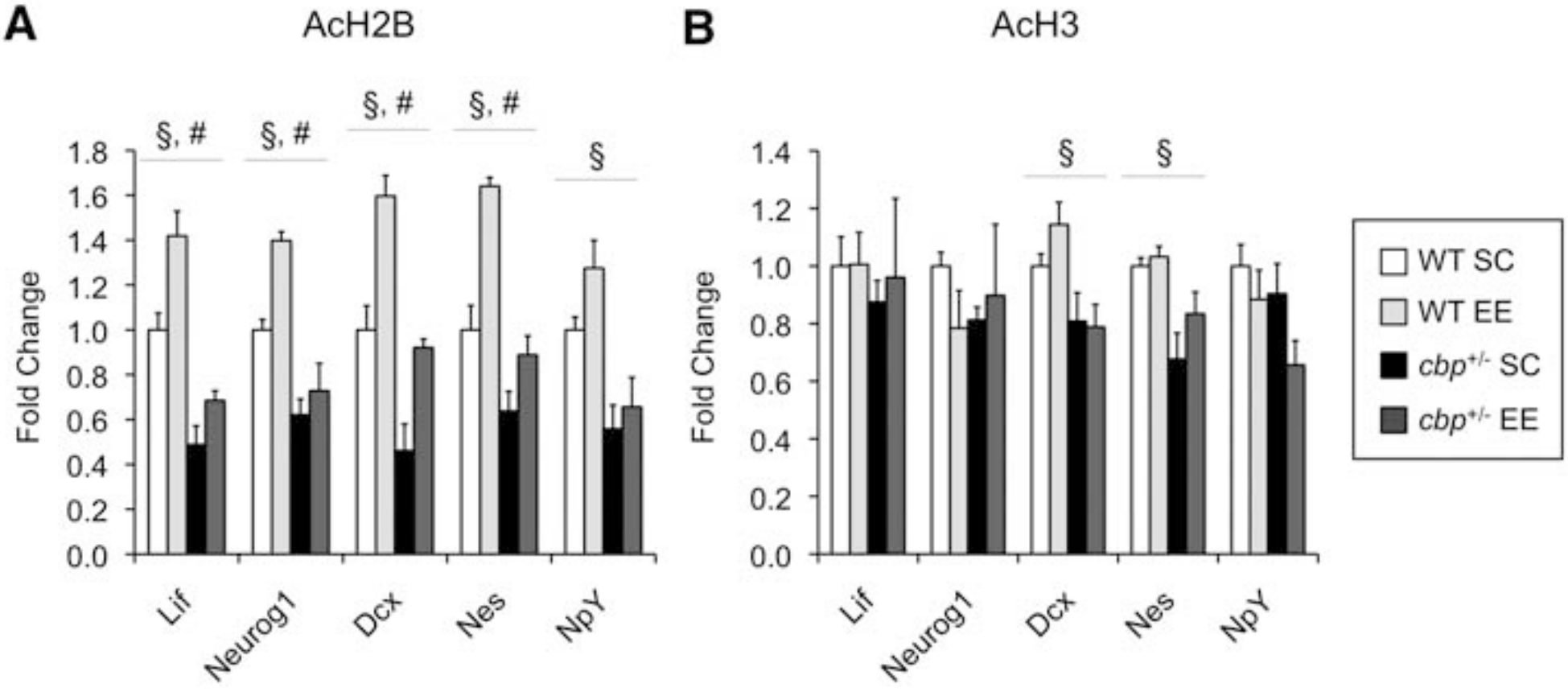
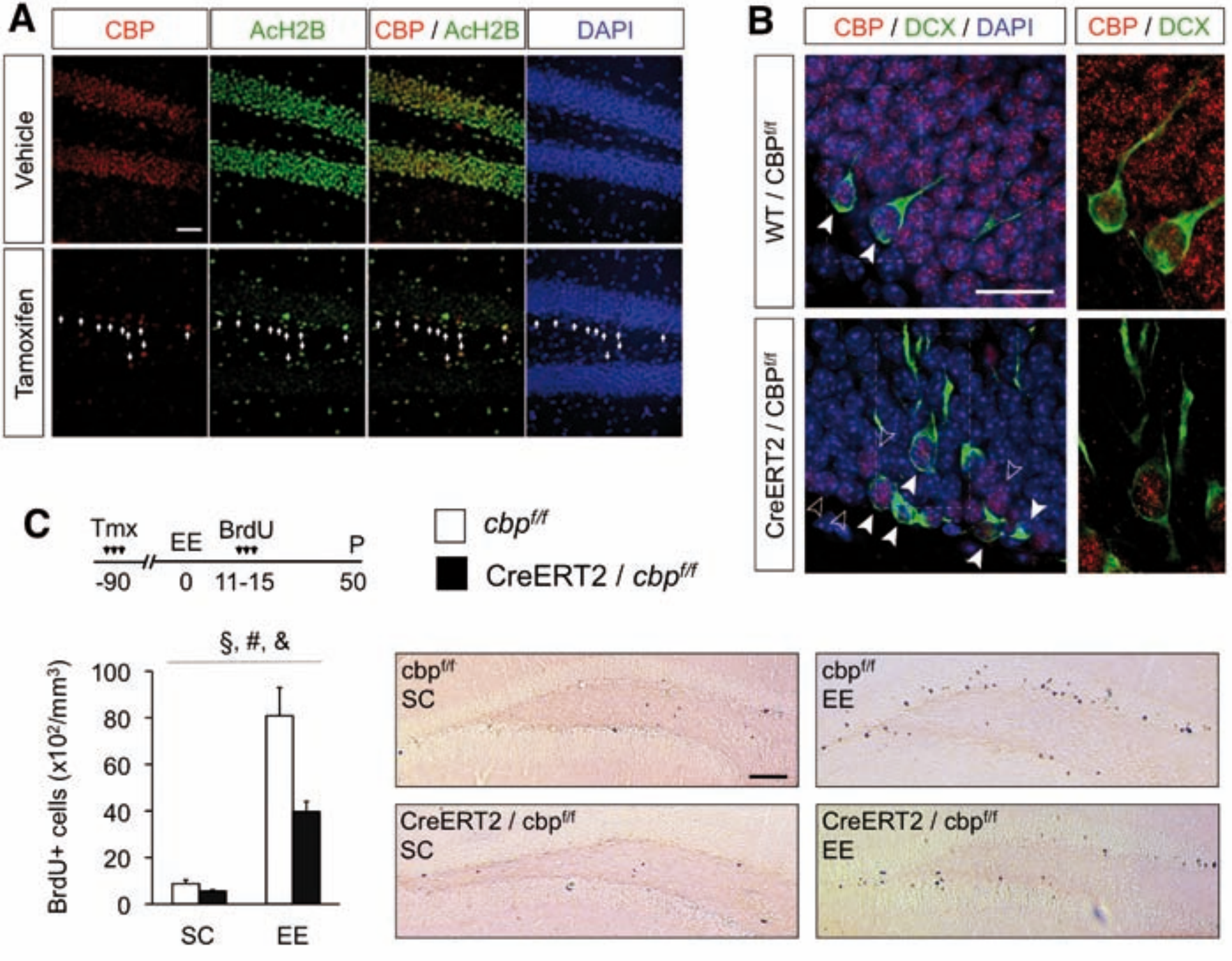

A
AcH2B
Fold Change
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
§, #
§, #
§, #
§, #
§
Lif
Neurog1
Dcx
Nes
NpY
B
AcH3
Fold Change
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
§
§
Lif
Neurog1
Dcx
Nes
NpY
WT SC
WT EE
cbp+/- SC
cbp+/- EE

Figure 8


A
CBP
AcH2B
CBP / AcH2B
DAPI
Vehicle
Tamoxifen
B
CBP / DCX / DAPI
CBP / DCX
WT / CBP f/f
CreERT2 / CBP f/f
C
Tmx
EE
BrdU
P
-90
0
11-15
50
cbp f/f
CreERT2 / cbp f/f
§, #, &
BrdU+ cells (x10²/mm³)
100
80
60
40
20
0
SC
EE
cbp f/f SC
cbp f/f EE
CreERT2 / cbp f/f SC
CreERT2 / cbp f/f EE