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Altered hippocampal expression of calbindin-D-28k and calretinin in $GABA_{B(1)}$-deficient mice

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Abstract

Balb/c $GABA_{B(1)}{}^{-/-}$ mice develop complex epileptiform activity, including spontaneous and audiogenic generalized seizures, 6–8 weeks after birth. The neuronal systems involved in these epilepsies have not been identified yet. Because the hippocampus is critically involved in epileptiform activity, we now investigated whether this brain region exhibits seizure-related alterations. Using semi-quantitative immunohistochemistry, we studied the temporal and cellular hippocampal expression pattern of two seizure-sensitive calcium-binding proteins, calbindin-D-28k and calretinin, in $GABA_{B(1)}{}^{-/-}$ mice. One month after birth, before the onset of overt epileptiform activity, wild-type (WT) and $GABA_{B(1)}{}^{-/-}$ mice exhibit comparable expression profiles for the two calcium-binding proteins. Three months after birth, once the epileptic phenotype is established, we observe clear alterations in the expression of calcium-binding proteins in the dentate gyrus area. $GABA_{B(1)}{}^{-/-}$ mice exhibit a 50% decline in the staining intensity of calbindin-D-28k expressing neurons and a 70% increase in the number of calretinin-positive neurons when compared to WT littermates. Six months after birth, the down-regulation of calbindin-D-28k protein is even more pronounced, while the calretinin expression in $GABA_{B(1)}{}^{-/-}$ mice reverts to the pattern seen in WT littermates. Our data demonstrate that the absence of functional $GABA_B$ receptors causes epileptiform activity through a mechanism that crucially involves dentate gyrus granule cells, and that this pathological activity is accompanied by adaptive changes.

Keywords: γ-Aminobutyric acid; $GABA_{B(1)}$; Epilepsy; Knock-out; Hippocampus; Calcium-binding proteins

1. Introduction

γ-Aminobutyric acid (GABA), the predominant inhibitory neurotransmitter in the mammalian central nervous system, signals through ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. $GABA_B$ receptors are coupled to G-proteins and modulate synaptic transmission by controlling neurotransmitter release and by causing postsynaptic hyperpolarization [1,2]. It is well known that an imbalance of excitatory to inhibitory synaptic transmission can cause epileptic seizures. In fact, many anti-convulsant drugs exert their therapeutic effect by enhancing GABAergic inhibition through a direct modulation of GABA metabolism or $GABA_A$ receptor function. Whether compounds that target $GABA_B$ receptors could be used to treat epilepsy is far from clear because of the paradoxical findings that $GABA_B$ agonists and antagonists can be both pro- and anti-convulsant, depending on the experimental model [3–8]. It was therefore interesting to examine whether a constitutive genetic loss of $GABA_B$ receptors would produce an epileptic phenotype. $GABA_{B(1)}{}^{-/-}$ and $GABA_{B(2)}{}^{-/-}$ mice were produced in a number of laboratories [9–12]. Because $GABA_B$ receptors are formed through the co-assembly of $GABA_{B(1)}$ with $GABA_{B(2)}$ subunits, all these mice essentially exhibit a complete lack of functional $GABA_B$ receptors, which consistently produces epileptiform activity.

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C57Bl6 or C57Bl6/129Sv $GABA_{B(1)}^{-/-}$ mice die within 3–4 weeks after birth, presumably due to the strong penetrance of seizure activity. Balb/c $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice are viable but suffer from spontaneous epileptic seizures that become apparent 6–8 weeks after birth [9,10]. It was shown that adult Balb/c $GABA_{B(1)}^{-/-}$ mice display a complex epileptic phenotype, characterized by frequent spontaneous clonic seizures and sporadic tonic-clonic and absence-type seizures [9]. Besides, $GABA_{B(1)}^{-/-}$ mice are very susceptible to audiogenic stimulation, which reliably precipitates tonic-clonic convulsions.

In order to address how the lack of $GABA_B$ receptors produces an epileptic phenotype in $GABA_{B(1)}^{-/-}$ mice, it is important to identify the brain regions and neuronal systems that are involved in seizure activity. Hippocampus and amygdala are two key structures implicated in convulsive generalized and temporal lobe seizures [13,14]. The prevalent seizures observed in $GABA_{B(1)}^{-/-}$ mice are therefore likely to involve limbic structures. Calbindin-D-28k (CB) and calretinin (CR) are two calcium-binding proteins that are often found to be up- or down-regulated in epilepsy. Intracellular Ca^{2+} plays a critical role in the generation and the establishment of epilepsy in the hippocampus [15] and therefore the expression of Ca^{2+}-binding proteins is frequently changed as a result of epileptic activity. In the hippocampus, CR and CB are predominantly expressed in subsets of interneurons and principal cells [16–18]. In order to know whether the epileptiform activity in $GABA_{B(1)}^{-/-}$ mice is associated with changes in the expression of known seizure-sensitive proteins in limbic structures, we analyzed the spatial expression pattern of CB and CR in the hippocampus of $GABA_{B(1)}^{-/-}$ mice before and after the onset of apparent epileptiform activity.

2. Materials and methods

2.1. Animals

All experiments conformed to Swiss legal requirements. Mice were killed at the age of 1 month (before the onset of overt seizures), 3 months (after the onset of seizures) and 6 months (advanced stage of epilepsy). All experimental mice were derived from breedings of Balb/c $GABA_{B(1)}^{+/-}$ mice [9]. Four pairs of knock-out and wild-type (WT) littermates were used for each stage analyzed.

2.2. Tissue preparation

The mice were anesthetized with isoflurane and then decapitated. The brains were immediately removed and fixed for 3 days at 4 °C by immersion into 4% freshly depolymerized paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Coronal 50 μm thick sections were cut using a vibroslicer (DTK-zero 1, DSK, Kyoto, Japan). The sections were collected in 0.02 M phosphate-buffered saline (PBS, pH 7.4) and stored at 4 °C.

2.3. Immunohistochemistry for calcium-binding proteins

The free-floating sections were rinsed for 30 min in 0.02 M PBS containing 2% H_2O_2 at room temperature. The sections were washed for 1 h in 0.02 M PBS containing 1% bovine serum albumin (BSA; Sigma, St. Louis, USA) and 0.1% Triton X-100, followed by incubation overnight at 4 °C with rabbit anti-calbindin (1:2000; Swant, Bellinzona, Switzerland) or rabbit anti-calretinin (1:2000; Swant) antibodies in PBS/BSA/Triton. The sections were rinsed twice for 15 min in PBS/BSA/Triton and once for 15 min in PBS. Detection of bound primary antibody was via the avidin–biotin complex system (Vector Laboratories, Burlingame, CA, UK). Briefly, the sections were first incubated for 2 h at room temperature with a biotinylated anti-rabbit antibody (1:400 in PBS), followed by a 1 h incubation with peroxidase-labeled avidin–biotin complex (1:100 avidin and 1:100 biotin). After several washes with PBS, the sections were stained with 0.05% 3,3′-diaminobenzidine (DAB; Sigma) in 0.05 M Tris pH 7.5 and 0.006% H_2O_2. The sections were finally slide-mounted and coverslipped for observation under a light microscope. Experiments without primary or secondary antibodies were performed as a control. A set of sections was stained with toluidine blue to compare the cytoarchitectonic organization of the hippocampus in WT and $GABA_{B(1)}^{-/-}$ mice.

2.4. Quantification and statistical analysis

Ten hippocampal sections per animal were examined through a Leica Dialux 20 transmission light microscope and pictures were captured with a color 3-CCD video camera (JVC KY-F55B). The images were digitalized under identical conditions (transmission light, camera voltage). Semi-quantification of CB- and CR-immunostaining intensity was performed in the CA1/2, CA3 and dentate gyrus regions using the Scion imaging software (Scion Corporation, Frederick, MD, USA). CR-immunopositive cells were counted manually in the left and right hemispheres of the dentate gyrus under the microscope. Results for WT and $GABA_{B(1)}^{-/-}$ mice were represented as percentage of WT ± S.E.M., compared by one-way ANOVA followed by a Scheffé's test. Changes were considered significant if $P < 0.05$.

3. Results

At all ages analyzed, we do not observe obvious morphological differences between the hippocampi of $GABA_{B(1)}^{-/-}$ mice and WT littermates, using toluidine blue staining (Fig. 1).

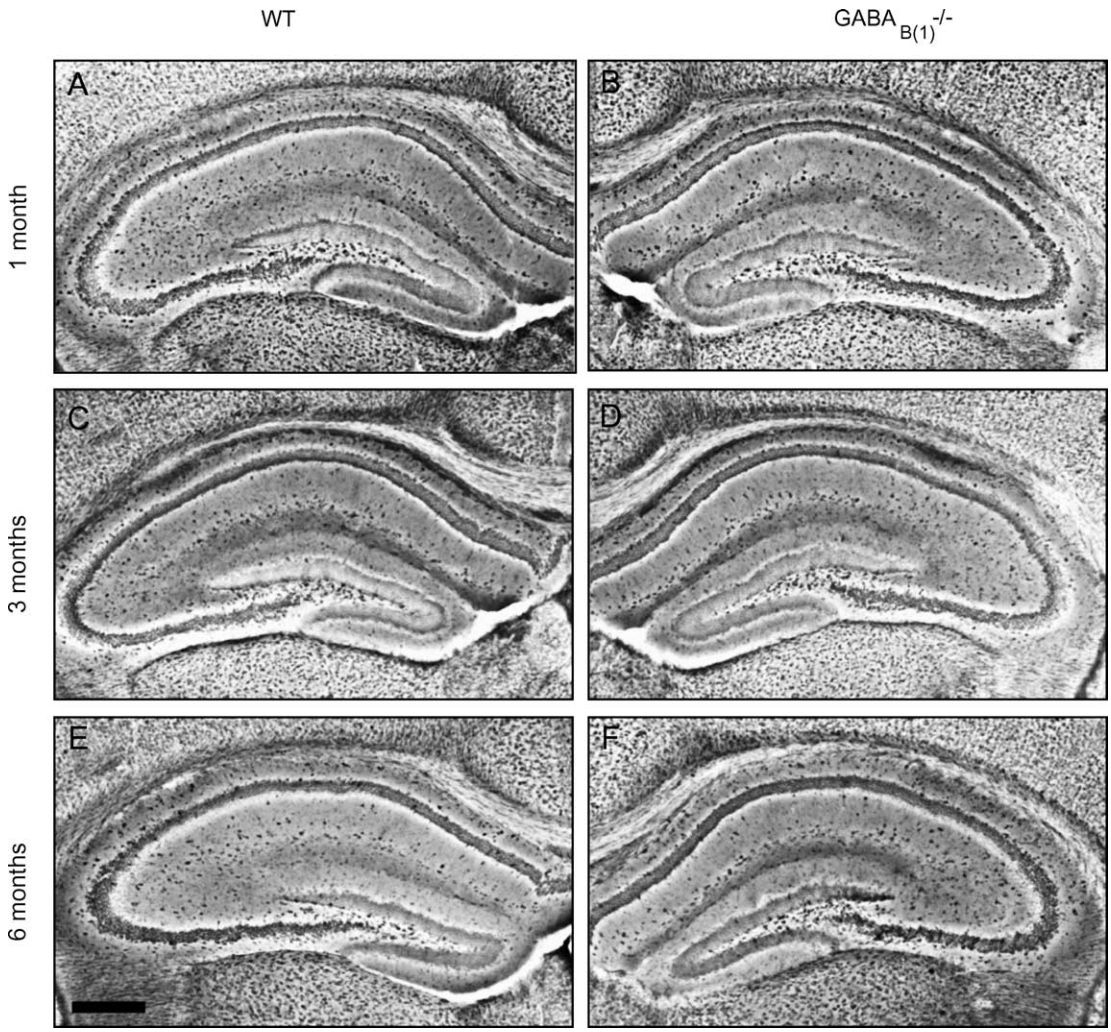

Fig. 1. Toluidine blue staining of coronal sections through the hippocampus of wild-type (WT) (A, C, E) and $GABA_{B(1)}^{-/-}$ mice (B, D, F) at 1 (A, B), 3 (C, D) or 6 (E, F) months of age. The cytoarchitecture of the hippocampus is identical in wild-type and $GABA_{B(1)}^{-/-}$ mice at all ages analyzed. Scale bar = 400 μm.

3.1. Calbindin-D-28k expression is down-regulated in the dentate gyrus of $GABA_{B(1)}^{-/-}$ mice

The CB expression pattern in the hippocampal formation of WT mice (Fig. 2A–E) agrees well with that described earlier [16,19,20]. We observe a marked differential distribution of CB-immunostaining between the dentate gyrus, CA3 and CA1/2 regions. In the dentate gyrus, CB-immunoreactivity localizes to the cell bodies and dendrites of granule cells, as well as to the mossy fibers projecting to the CA3 area. No immunoreactive cells are seen in the CA3 pyramidal cell layer. A few intensely stained cells are observed in the stratum radiatum/lacunosum region of the CA3 area, most likely corresponding to interneurons. In the CA1/2 area, CB-immunostaining is present mainly in the pyramidal cell bodies and some scattered interneurons (arrow in Fig. 2A), as well as in the Schaffer collaterals. While 1-month-old $GABA_{B(1)}^{-/-}$ mice exhibit a similar CB-staining pattern and intensity as WT mice throughout the hippocampus (Fig. 2A and B), $GABA_{B(1)}^{-/-}$ mice of 3 and 6 months of age exhibit a significant decrease of the CB-staining intensity in specific areas of the hippocampal formation, following a spatio-temporal pattern (Fig. 2C–F). Concomitant with the development of epileptic seizures, $GABA_{B(1)}^{-/-}$ mice exhibit a significant decrease of the CB-staining intensity in dentate gyrus granule cells (Fig. 2C–F). The decrease in staining intensity is approximately 50% ($P < 0.001$) and 70% ($P < 0.001$) at 3 and 6 months of age, respectively (Table 1). In 3-month-old $GABA_{B(1)}^{-/-}$ mice, the reduced expression of CB in the granule cell bodies does not result in a decline in the intensity of CB-immunoreactivity in the mossy fibers (Fig. 2C and D). However, in 6-month-old $GABA_{B(1)}^{-/-}$ mice, a drastic 80% ($P < 0.0005$) decline in the intensity of CB-immunoreactivity is seen in the mossy fibers compared to WT mice (Fig. 2E and F; Table 1). At all ages analyzed, no significant difference in CB-immunoreactivity is observed in the CA1/2 area of $GABA_{B(1)}^{-/-}$ mice as compared to WT littermate mice (Fig. 2A–F).

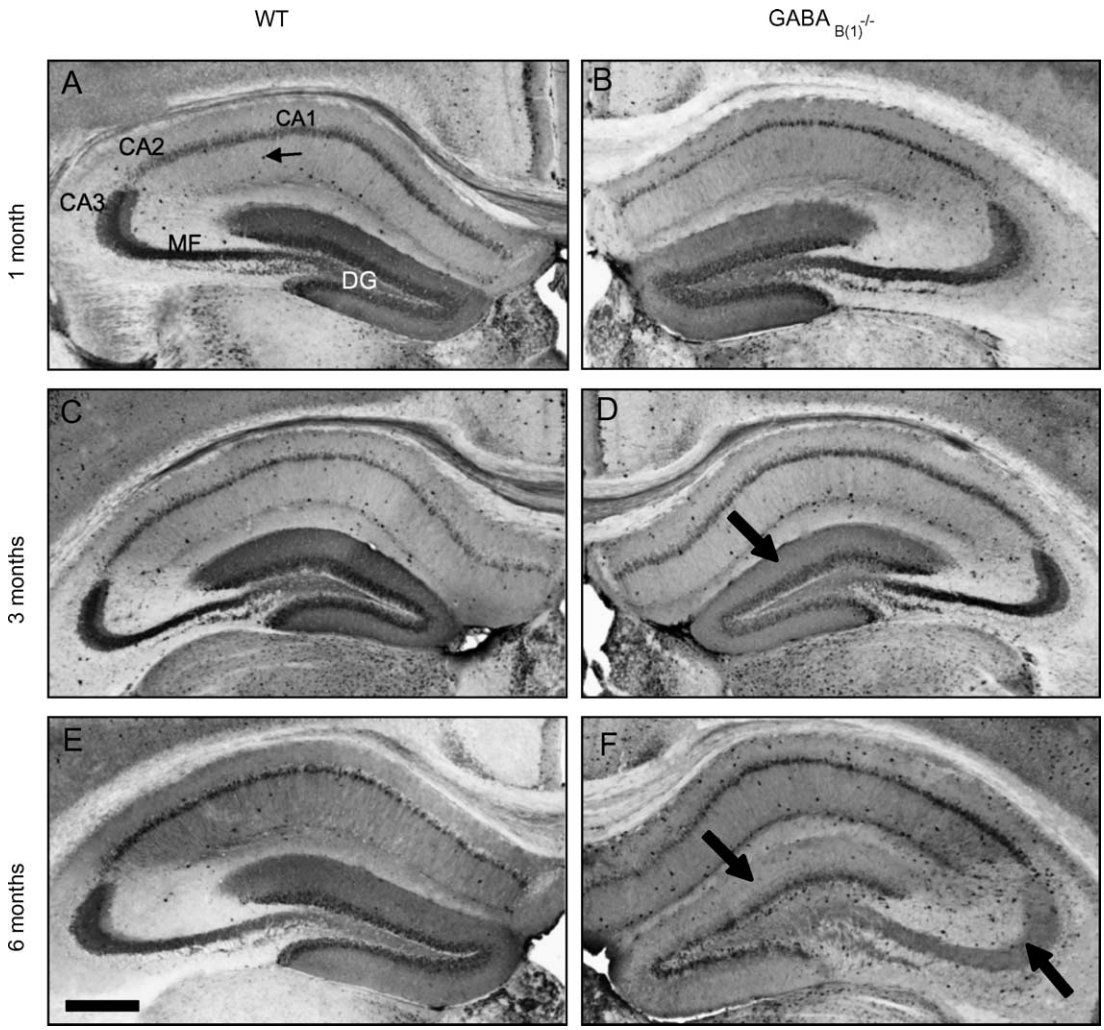

Fig. 2. CB-immunoreactivity in coronal sections through the hippocampus of WT (A, C, E) and $GABA_{B(1)}^{-/-}$ mice (B, D, F) at 1 (A, B), 3 (C, D) or 6 (E, F) months of age. While the pattern of CB-immunoreactivity is not altered in 1-month-old $GABA_{B(1)}^{-/-}$ mice, we observe a decline in CB staining intensity in the dentate gyrus of 3-month-old $GABA_{B(1)}^{-/-}$ vs. WT mice (arrow in D). In 6-month-old animals, this decrease in CB-immunoreactivity is also seen in the mossy fibers and is accentuated in the dentate gyrus (arrows in F). DG, dentate gyrus; MF, mossy fibers. Scale bar = 400 μm.

3.2. $GABA_{B(1)}^{-/-}$ mice exhibit a transient increase in calretinin-positive neurons

The immunohistochemical CR-staining pattern that we observe in the dentate gyrus of WT mice agrees well with earlier data [21,22]. In WT mice, we detect CR-immunoreactivity in a few faintly stained infragranular granule cells (at the interface of the hilus; arrow in Fig. 3A), as well as in few scattered interneurons (arrowhead in Fig. 3A). In addition, hilar mossy cells are weakly immunoreactive for CR while their projections to the inner molecular layer (IML) are strongly stained (Fig. 3A). No difference in

Table 1
Semi-quantitative analysis of changes in the staining intensity of calbindin-D-28k and in the number of calretinin-immunopositive neurons in the hippocampus of WT and $GABA_{B(1)}^{-/-}$ mice

Marker	Area	1 month		3 months		6 months	
		WT	KO	WT	KO	WT	KO
CB	DG	100 ± 10	123 ± 13	100 ± 7	51 ± 11**	100 ± 18	31 ± 9**
	MF	100 ± 8	103 ± 8	100 ± 7	93 ± 11	100 ± 16	16 ± 3***
	CA1/2	100 ± 14	117 ± 19	100 ± 13	81 ± 9	100 ± 11	89 ± 14
CR	DG	100 ± 10	98 ± 5	100 ± 5	170 ± 30*	100 ± 10	101 ± 5

CB, calbindin; CR, calretinin; MF, mossy fibers. The data are represented as mean percentage (±S.E.M.) of the WT value.
* $P < 0.05$ as compared with the WT value using a one-way ANOVA followed by a Scheffé's test.
** $P < 0.001$ as compared with the WT value using a one-way ANOVA followed by a Scheffé's test.
*** $P < 0.0005$ as compared with the WT value using a one-way ANOVA followed by a Scheffé's test.

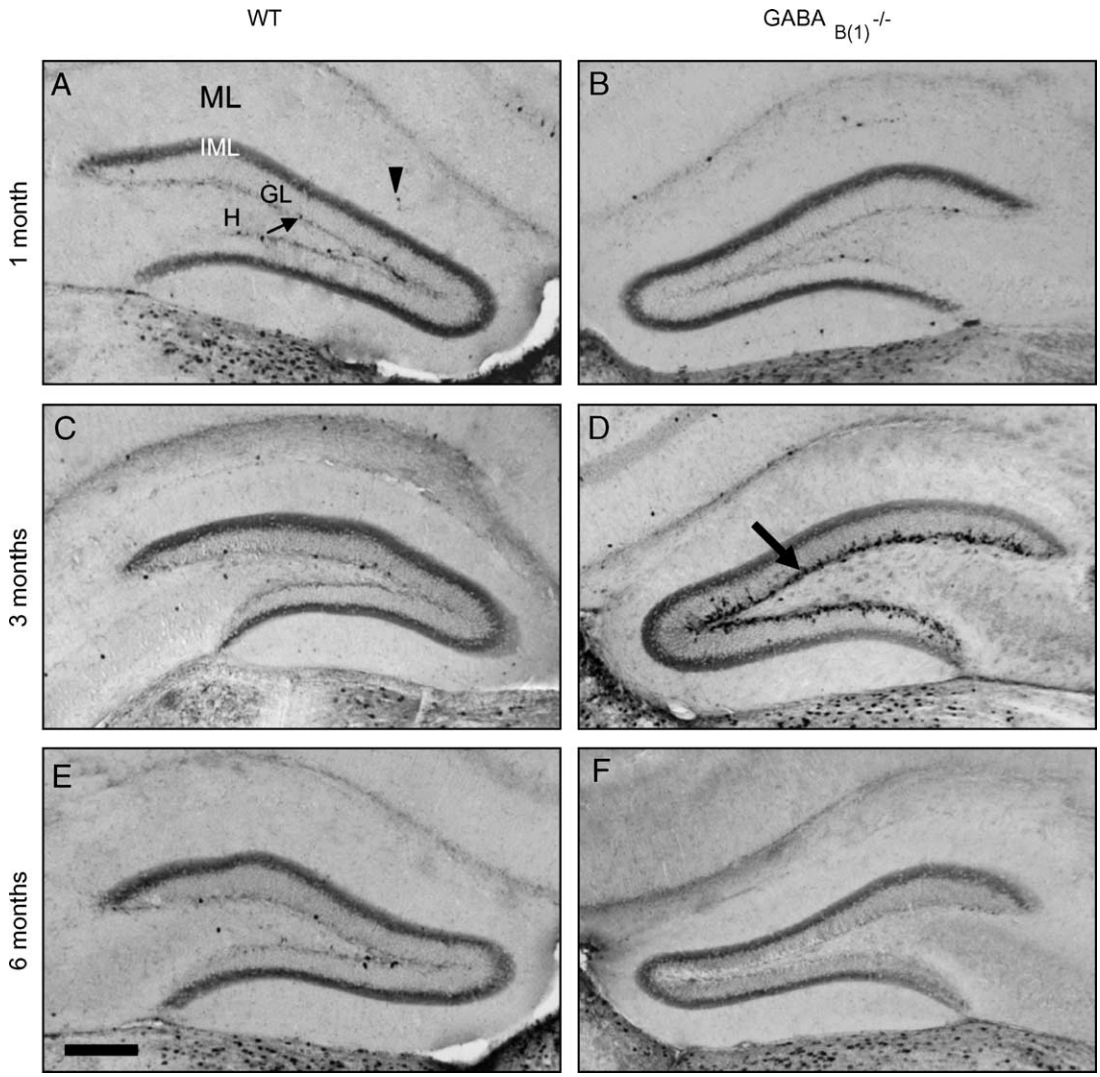

Fig. 3. CR-immunoreactivity in coronal sections through the dentate gyrus of WT (A, C, E) and $GABA_{B(1)}^{-/-}$ mice (B, D, F) at 1 (A, B), 3 (C, D) or 6 (E, F) months of age. $GABA_{B(1)}^{-/-}$ mice exhibit a transient increase in the number of CR-positive neurons in the subgranular zone of the dentate gyrus at the age of 3 months (arrow in D). GL, granule cell layer; H, hilus; IML, inner molecular layer; ML, molecular layer. Scale bar = 300 μm.

CR-immunoreactivity is observed between 1-month-old $GABA_{B(1)}^{-/-}$ mice and WT littermates (Fig. 3A and B). However, in 3-month-old mice, a transient 70% increase in the number of immunoreactive infragranular neurons is seen in $GABA_{B(1)}^{-/-}$ versus WT mice ($P < 0.05$; Fig. 3C and D; Table 1). These neurons are strongly immunoreactive and exhibit an intensely stained dendritic arborisation. In 6-month-old $GABA_{B(1)}^{-/-}$ mice the pattern of CR-immunoreactivity reverts to the one seen in WT mice with the presence of rare CR-immunopositive neurons in the infragranular zone of the dentate gyrus (Fig. 3E and F).

4. Discussion

In this study we use two known seizure-sensitive calcium-binding proteins, CB and CR, to identify neuronal populations that are involved in the generation and/or progression of seizure activity in $GABA_{B(1)}^{-/-}$ mice. Our results indicate that the hippocampal formation, in particular the dentate gyrus, exhibits changes concomitant with the development of seizure activity.

4.1. Relation to $GABA_B$ receptor expression

Our results demonstrate that the dentate gyrus of $GABA_{B(1)}^{-/-}$ mice exhibits changes in the expression pattern of calcium-binding proteins, and that these alterations take place with or shortly after the onset of epileptic seizure activity. The granule cells of the dentate gyrus are ideally located for initiating and spreading of epileptic seizures because their projections, the mossy fibers, represent the major input to the CA3 area. In the dentate gyrus, $GABA_B$ receptors are mostly detected in the molecular layer, which is contacted by the perforant pathway and inhibitory dentate hilar cells. $GABA_B$ receptors are expressed to a lesser extent in the hilus and almost absent in granule cell bodies. In addition, it has been shown that

$GABA_B$ receptors are expressed presynaptically on mossy fibers [23], where they restrict glutamate release from the terminals. This is in line with experiments in brain slices of C57Bl6 $GABA_{B(1)}^{-/-}$ mice where the lack of $GABA_B$ receptors resulted in increased NMDA-dependent excitability in the CA3 field [24]. Neither CB nor CR is normally expressed in CA3 pyramidal cells. Therefore, any seizure-related changes in CA3 neurons would have gone undetected using CB and CR immunohistochemistry.

4.2. Calbindin-D-28k expression is down-regulated in the dentate gyrus of $GABA_{B(1)}^{-/-}$ mice

At 1 month of age, prior to the onset of apparent epileptic seizures, the expression pattern of CB is unchanged in $GABA_{B(1)}^{-/-}$ mice compared to WT littermates. However, after 3 months CB-immunoreactivity is significantly decreased in granule cells of the dentate gyrus, which is followed by a delayed decrease in CB-immunoreactivity in the mossy fibers in the CA3 area. A decrease in CB-immunoreactivity in these brain regions has been reported in other models of epilepsy as well as in patients that suffer from mesial temporal lobe epilepsy [25–27]. Ca^{2+} has been shown to inhibit the transcription of the CB gene [28]. Therefore, the decrease in CB protein levels in the dentate granule cells of $GABA_{B(1)}^{-/-}$ mice may be attributed to an increased Ca^{2+} influx as a result of increased presynaptic glutamate release and/or reduced postsynaptic inhibition. However, Sonnenberg et al. have demonstrated that a decrease in CB protein amounts is not necessarily correlated with a decrease in CB mRNA, suggesting that the translation and/or the degradation of CB are also regulated during epilepsy [29]. This may be a reason why in $GABA_{B(1)}^{-/-}$ mice, the decline in CB contents is delayed in the mossy fibers as compared with the granule cell layer. The loss of CB in granule cells may, in turn, lead to a protective inactivation of high-voltage activated L-type Ca^{2+} channels [30–33]. The fact that the decrease in CB expression affects the dentate gyrus but not the CA1/2 fields could be due to a differential control of Ca^{2+} homeostasis. Indeed, it has been shown that epilepsy or ischemic insults can result in higher increase of Ca^{2+} influx in the dentate granule cells than in CA1 pyramidal cells [34–37].

4.3. Calretinin expression is transiently up-regulated in the dentate gyrus of $GABA_{B(1)}^{-/-}$ mice

The absence of functional $GABA_B$ receptors in $GABA_{B(1)}^{-/-}$ mice leads to a transient but impressive increase in the number of CR-positive neurons in the subgranular layer of the dentate gyrus. Such an increase has been observed in several animal models of epilepsy as well as in patients suffering from temporal lobe epilepsy [38]. The detection of CR in granule cells of the dentate gyrus may reflect the presence of newly generated neurons that are formed as a consequence of the epileptic seizures [21,39–41]. Furthermore, the temporary expression of CR in the dentate gyrus of $GABA_{B(1)}^{-/-}$ mice at the age of 3 months is in accordance with previous reports demonstrating that CR is a transient marker for maturing granule cells in adult dentate gyrus [40]. After maturation, these newly generated neurons integrate slowly into the hippocampal circuitry [42], mature into functional neurons that exhibit some of the morphological and electrophysiological properties of granule cells [43] and develop a strong afferent input [44]. Besides, these CR-positive neurons could become CB-positive after maturation [40,45]. The stimulated hippocampal neurogenesis observed in a variety of models for epilepsy is expected to reflect an adaptive process to cell death, which particularly affects the granule cell population [46,47]. However, the fact that we and others [48–51] found no evidence for cell death in the granule cell layer in parallel to epilepsy seizures does not support this hypothesis.

In conclusion, our study identifies the dentate gyrus as a likely structure to be involved in the generation and/or maintenance of the seizure activity observed in Balb/c $GABA_{B(1)}^{-/-}$ mice. However, it is reasonable to expect that additional brain regions are involved in generating and propagating of seizures in these mice.

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References

[1] Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of $GABA_B$ receptors. Physiol Rev 2004;84:835–67.
[2] Calver AR, Davies CH, Pangalos M. $GABA_B$ receptors: from monogamy to promiscuity. Neurosignals 2002;11(6):299–314.
[3] Karlsson G, Kolb C, Hausdorf A, Portet C, Schmutz M, Olpe HR. $GABA_B$ receptors in various in vitro and in vivo models of epilepsy: a study with the $GABA_B$ receptor blocker CGP 35348. Neuroscience 1992;47(1):63–8.
[4] Mangan PS, Lothman EW. Profound disturbances of pre- and postsynaptic $GABA_B$ receptor-mediated processes in region CA1 in a chronic model of temporal lobe epilepsy. J Neurophysiol 1996; 76(2):1282–96.
[5] Scanziani M. GABA spillover activates postsynaptic $GABA_B$ receptors to control rhythmic hippocampal activity. Neuron 2000;25(3): 673–81.
[6] Chandler KE, Princivalle AP, Fabian-Fine R, Bowery NG, Kullmann DM, Walker MC. Plasticity of $GABA_B$ receptor-mediated heterosynaptic interactions at mossy fibers after status epilepticus. J Neurosci 2003;23(36):11382–91.

[7] Vacher CM, Bettler B. $GABA_B$ receptors as potential therapeutic targets. Curr Drug Target CNS Neurol Disord 2003;2(4):248–59.
[8] Depaulis A, Deransart C, Vergnes M, Marescaux C. GABAergic mechanisms in generalized epilepsies: the neuroanatomical dimension. Rev Neurol Paris 1997;153(Suppl 1):S8–S13.
[9] Schuler V, Luscher C, Blanchet C, Klix N, Sansig G, Klebs K, et al. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic $GABA_B$ responses in mice lacking $GABA_{B(1)}$. Neuron 2001;31(1):47–58.
[10] Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, et al. Redistribution of $GABA_{B(1)}$ protein and atypical $GABA_B$ responses in $GABA_{B(2)}$-deficient mice. J Neurosci 2004;24(27):6086–97.
[11] Queva C, Bremner-Danielsen M, Edlund A, Ekstrand AJ, Elg S, Erickson S, et al. Effects of GABA agonists on body temperature regulation in $GABA_{B(1)}^{-/-}$ mice. Br J Pharmacol 2003;140(2):315–22 (Epub 2003 August 11).
[12] Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, et al. Epileptogenesis and enhanced prepulse inhibition in $GABA_{B(1)}$-deficient mice. Mol Cell Neurosci 2001;17(6):1059–70.
[13] Johnston MV. Developmental aspects of epileptogenesis. Epilepsia 1996;37(Suppl 1):S2–9.
[14] Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. Ann Neurol 1994;35(6):640–54.
[15] Avanzini G, Franceschetti S. Cellular biology of epileptogenesis. Lancet Neurol 2003;2(1):33–42.
[16] Baimbridge KG, Miller JJ. Immunohistochemical localization of calcium-binding protein in the cerebellum, hippocampal formation and olfactory bulb of the rat. Brain Res 1982;245(2):223–9.
[17] Resibois A, Rogers JH. Calretinin in rat brain: an immunohistochemical study. Neuroscience 1992;46(1):101–34.
[18] Freund TF, Buzsaki G. Interneurons of the hippocampus. Hippocampus 1996;6(4):347–470.
[19] Celio MR. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 1990;35(2):375–475.
[20] Jinno S, Kosaka T. Patterns of expression of calcium binding proteins and neuronal nitric oxide synthase in different populations of hippocampal GABAergic neurons in mice. J Comp Neurol 2002;449(1):1–25.
[21] Liu Y, Fujise N, Kosaka T. Distribution of calretinin immunoreactivity in the mouse dentate gyrus. I. General description. Exp Brain Res 1996;108(3):389–403.
[22] Fujise N, Liu Y, Hori N, Kosaka T. Distribution of calretinin immunoreactivity in the mouse dentate gyrus: II. Mossy cells, with special reference to their dorsoventral difference in calretinin immunoreactivity. Neuroscience 1998;82(1):181–200.
[23] Vogt KE, Nicoll RA. Glutamate and gamma-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. Proc Natl Acad Sci USA 1999;96(3):1118–22.
[24] Brown JT, Gill CH, Farmer CE, Lanneau C, Randall AD, Pangalos MN, et al. Mechanisms contributing to the exacerbated epileptiform activity in hippocampal slices of $GABA_{B(1)}$ receptor subunit knockout mice. Epilepsy Res 2003;57(2/3):121–36.
[25] Baimbridge KG, Miller JJ. Hippocampal calcium-binding protein during commissural kindling-induced epileptogenesis: progressive decline and effects of anticonvulsants. Brain Res 1984;324(1):85–90.
[26] Yang Q, Wang S, Hamberger A, Celio MR, Haglid KG. Delayed decrease of calbindin immunoreactivity in the granule cell-mossy fibers after kainic acid-induced seizures. Brain Res Bull 1997;43(6):551–9.
[27] Wittner L, Eross L, Szabo Z, Toth S, Czirjak S, Halasz P, et al. Synaptic reorganization of calbindin-positive neurons in the human hippocampal CA1 region in temporal lobe epilepsy. Neuroscience 2002;115(3):961–78.
[28] Arnold DB, Heintz N. A calcium responsive element that regulates expression of two calcium binding proteins in Purkinje cells. Proc Natl Acad Sci USA 1997;94(16):8842–7.
[29] Sonnenberg JL, Frantz GD, Lee S, Heick A, Chu C, Tobin AJ, et al. Calcium binding protein (calbindin-D28k) and glutamate decarboxylase gene expression after kindling induced seizures. Brain Res Mol Brain Res 1991;9(3):179–90.
[30] Mody I, Reynolds JN, Salter MW, Carlen PL, MacDonald JF. Kindling-induced epilepsy alters calcium currents in granule cells of rat hippocampal slices. Brain Res 1990;531(1/2):88–94.
[31] Kohr G, Lambert CE, Mody I. Calbindin-D28K (CaBP) levels and calcium currents in acutely dissociated epileptic neurons. Exp Brain Res 1991;85(3):543–51.
[32] Beck H, Steffens R, Heinemann U, Elger CE. Ca^{2+}-dependent inactivation of high-threshold Ca^{2+} currents in hippocampal granule cells of patients with chronic temporal lobe epilepsy. J Neurophysiol 1999;82(2):946–54.
[33] Nagerl UV, Mody I, Jeub M, Lie AA, Elger CE, Beck H. Surviving granule cells of the sclerotic human hippocampus have reduced Ca^{2+} influx because of a loss of calbindin-D(28k) in temporal lobe epilepsy. J Neurosci 2000;20(5):1831–6.
[34] Krnjevic K, Leblond J. Anoxia reversibly suppresses neuronal calcium currents in rat hippocampal slices. Can J Physiol Pharmacol 1987;65(10):2157–61.
[35] Tsubokawa H, Oguro K, Robinson HP, Masuzawa T, Kirino T, Kawai N. Abnormal Ca^{2+} homeostasis before cell death revealed by whole cell recording of ischemic CA1 hippocampal neurons. Neuroscience 1992;49(4):807–17.
[36] Connor JA, Razani-Boroujerdi S, Greenwood AC, Cormier RJ, Petrozzino JJ, Lin RC. Reduced voltage-dependent Ca^{2+} signaling in CA1 neurons after brief ischemia in gerbils. J Neurophysiol 1999;81(1):299–306.
[37] Gorter JA, Borgdorff AJ, van Vliet EA, Lopes da Silva FH, Wadman WJ. Differential and long-lasting alterations of high-voltage activated calcium currents in CA1 and dentate granule neurons after status epilepticus. Eur J Neurosci 2002;16(4):701–12.
[38] Blumcke I, Beck H, Suter B, Hoffmann D, Fodisch HJ, Wolf HK, et al. An increase of hippocampal calretinin-immunoreactive neurons correlates with early febrile seizures in temporal lobe epilepsy. Acta Neuropathol Berl 1999;97(1):31–9.
[39] Gray WP, Sundstrom LE. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. Brain Res 1998;790(1/2):52–9.
[40] Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, et al. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci 2003;24(3):603–13.
[41] Liu S, Wang J, Zhu D, Fu Y, Lukowiak K, Lu YM. Generation of functional inhibitory neurons in the adult rat hippocampus. J Neurosci 2003;23(3):732–6.
[42] Overstreet LS, Hentges ST, Bumaschny VF, de Souza FS, Smart JL, Santangelo AM, et al. A transgenic marker for newly born granule cells in dentate gyrus. J Neurosci 2004;24(13):3251–9.
[43] van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature 2002;415(6875):1030–4.
[44] Scharfman HE, Sollas AE, Berger RE, Goodman JH, Pierce JP. Perforant path activation of ectopic granule cells that are born after pilocarpine-induced seizures. Neuroscience 2003;121(4):1017–29.
[45] Wouterlood FG, Grosche J, Hartig W. Co-localization of calretinin and calbindin in distinct cells in the hippocampal formation of the rat. Brain Res 2001;922(2):310–4.
[46] Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc Natl Acad Sci USA 1997;94(19):10432–7.
[47] Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by

seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci 1997;17(10):3727–38.

[48] Holmes GL, Gairsa JL, Chevassus-Au-Louis N, Ben-Ari Y. Consequences of neonatal seizures in the rat: morphological and behavioral effects. Ann Neurol 1998;44(6):845–57.

[49] Scott BW, Wang S, Burnham WM, De Boni U, Wojtowicz JM. Kindling-induced neurogenesis in the dentate gyrus of the rat. Neurosci Lett 1998;248(2):73–6.

[50] Nakagawa E, Aimi Y, Yasuhara O, Tooyama I, Shimada M, McGeer PL, et al. Enhancement of progenitor cell division in the dentate gyrus triggered by initial limbic seizures in rat models of epilepsy. Epilepsia 2000;41(1):10–8.

[51] Sankar R, Shin D, Liu H, Katsumori H, Wasterlain CG. Granule cell neurogenesis after status epilepticus in the immature rat brain. Epilepsia 2000;41(Suppl 6):S53–6.