IMPAIRED DENDRITIC SPINE MATURATION IN GABA$_A$ RECEPTOR α1 SUBUNIT KNOCK OUT MICE

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Abstract—In this study we investigated the functional implications of GABA$_A$ receptor α1 subunit deletion on dendritic arborization and spine maturation in the visual cortex. This subunit is normally strongly upregulated during early postnatal development. Our main finding is that mice lacking the GABA$_A$ receptor α1 subunit displayed an increased density of dendritic filopodia during the second and third postnatal weeks. However, there was a concomitant decreased density of mature mushroom-shaped spines, which became more pronounced in adults. In contrast, dendritic arborization was not altered in these mice. We propose that an increased efficacy of the inhibitory synaptic transmission in the α1 knock out mice may lead to an enhancement of the outgrowth of filopodia around eye opening, but to a failure in spine maturation at later stages. © 2003 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: visual cortex, development, synaptic transmission, morphology, critical period.

In rodent visual cortex, developmental changes in GABA$_A$ receptor composition and function occur around eye opening (Araki et al., 1992; Bosman et al., 2002; Fritschy et al., 1994; Laurie et al., 1992), preceding the critical period when neuronal connections are fine tuned. Thus far, functional implications of the alterations in GABA$_A$ receptor expression and properties on maturation of the cortical cytoarchitecture have not been addressed. In this study,

we investigated dendritic arborization and spine maturation in the α1 knock out (KO) mice. These mice lack the subunit, which is normally strongly upregulated in the neocortex during development. The α1 KO mice display a lengthening of the synaptic current decay, indicating that the α1 subunit is an important causal factor of the developmental decrease in current decay (Vicini et al., 2001). For the present study we used the α1 KO mice from another laboratory (Sur et al., 2001). In these mice, the synaptic efficacy of GABAergic transmission was enhanced, partly due to the increased synaptic current decay and partly due to an enlargement of the current amplitude of miniature inhibitory postsynaptic currents (Bosman et al., 2002). It was expected that this would have major implications on neuronal activity, and therefore on activity dependent events including neurite outgowth (Kalb, 1994; Vogel and Prittie, 1995) and spine maturation (Dalva et al., 1994; Drakew et al., 1999; Kossel et al., 1997; McKinney et al., 1999; Rocha and Sur, 1995). In this study we measured the dendritic arborization in adult α1 KO mice and WT mice to test whether morphological maturation is affected in the α1 KO mice. In addition, we studied both the density of filopodia and mushroom-shaped spines at different stages of development in α1 KO and wild type (WT) mice. To investigate the consequences of the absence of the α1 subunit, we specifically focused on time points in development when, in WT mice, α1 subunit upregulation has just started (postnatal day [pn]14) or has reached its plateau level (pn21). Finally the ultimate consequences concerning spine density were studied in adult mice. To test whether compensatory regulation has occurred in α1 KO mice regarding other GABA$_A$ receptor α subunits or growth factors, quantitative polymerase chain reaction (PCR) experiments were conducted.

EXPERIMENTAL PROCEDURES

Animals

WT and mutant lacking the α1 subunit of the GABA$_A$ receptor were used in a mixed 50% C57BL6–50% 129SvEv background, as previously described (Sur et al., 2001). For this study F6 generation mice have been used of either pn14, pn21 or adult age (3 months). All experiments were approved by the Local Committee of Animal Welfare, thereby following the international standards for animal care taking and handling during experiments. The number of animals used and their suffering was kept to a minimum.

Rapid Golgi staining

Mice were decapitated, the brains were quickly removed and fixed in a freshly made solution of 2% glutaraldehyde and kept in the

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dark overnight at room temperature. The brains were briefly rinsed in distilled water and placed in 2% potassium dichromate–1% osmium tetroxide (4 parts to 1, respectively) for 3–6 days in the dark at room temperature. The pieces were washed with 0.75% silver nitrate until the red precipitate could no longer be seen in the wash and then transferred to fresh silver nitrate and kept for 3–6 days in the dark at room temperature. Subsequently, the brains were transferred to 70% ethanol and 160 μm sections were cut from the visual cortex on a vibratome. The sections were placed on subbed slides, dehydrated, cleared in Histoclear (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) and mounted in Histomount (Sigma-Aldrich Chemie B.V.) and assigned codes that did not reveal group identity.

## Dendritic morphology and spine density quantification

After Golgi staining (see Fig. 1A), pyramidal (Fig. 1B) and nonpyramidal cells (Fig. 1C) in layers II, III or IV of the visual cortex were analyzed. These superficial layers were chosen because incoming information from the lateral geniculate nucleus is processed here. Cells to be analyzed were chosen in the middle of the section to avoid the encountering of cut endings. Cells with damaged dendrites were not included in the measurements. Apical dendrites were defined as single, thick dendrites that extended from the dorsal aspect of the neuronal soma toward the pial surface; basal dendrites were defined as those extending radially from the soma (see Fig. 1B).

Golgi-stained neurons were morphologically reconstructed using a semi-automated dendrite measuring system (Overdijk et al., 1978; Uylings et al., 1986). We have quantitatively measured the following parameters: (i) total dendritic length per neuron; (ii) total apical dendritic length; (iii) total basal dendritic length. Total dendritic length measurements were further analyzed as follows: (a) path length (length of dendritic path from soma to a terminal tip); (b) intermediate segment length (segment lengths between two consecutive branch points); (c) terminal segment length (segment length between a final branch point and terminal tip); (d) number of dendritic segments per neuron; and (e) number of tips per dendrite. Each morphological parameter was statistically tested for possible differences between the α1 KO and the WT group using the non-parametric Mann-Whitney test.

In order to quantify spine density, the numbers of spines along a distance of 10 μm at the first intermediate segment length from the cell soma on both basal and apical dendrites were counted. A discrimination was made between filopodia-like protrusions and mushroom-shaped spines. Uniform thin protrusions were counted as filopodia, whereas thin-necked and thick-headed protrusions were counted as mushroom-shaped spines (Harris and Kater, 1994). In those cases where the total length of the spine could not be adequately seen, protrusions were excluded from analysis. Possible differences between the α1 KO and the WT group were statistically tested using the non-parametric Kruskal-Wallis test. All data collection and analysis was performed by an observer blinded to the genotype of the mice.

Quantitative PCR

Specific primer combinations (Eurogentec) were designed for each transcript. Amplified sequences were: brain-derived growth factor (BDNF; forward primer: G C T G G A T G A G G A C C A G A A G G T; reverse primer: G A G G C T C C A A A G G C A C T T G A), GABA receptor type A (GABA$_A$R) α1 NM_010250.1 (nucleotide [nt] 466–562); GABA$_A$R α2 NM_008066.1 (nt 1557–1635); GABA$_A$R α3 NM_008067.1 (nt 1549–1633); GABA$_A$R α4 gi 12851204 (nt 1776–1858); GABA$_A$R α5 (forward primer: AAAAGACATACAACAGCATCAGCAA; reverse primer: GAAAGTGCCAAACAAGATGGG), postsynaptic density protein 95 (forward primer: GAAGCTGGAGCAGGAGTTCAC; reverse
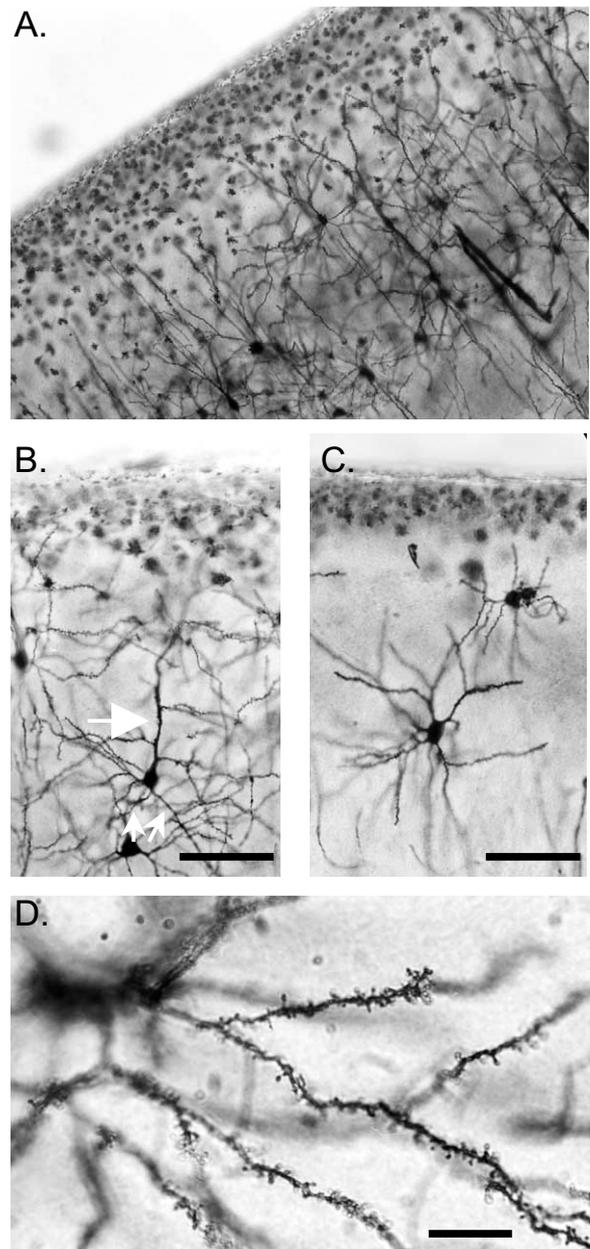
Fig. 1. Golgi-impregnated neurons in the visual cortex. Examples of Golgi-impregnated visual cortex tissue of adult α1 KO mice. (A) Overview of all cortical layers. Pial surface facing toward the left upward corner. (B) Enlargement of layer IV pyramidal cell, showing an apical dendrite projecting toward the pial surface (heavy arrow) and basal dendrites (thin arrow). Bar=100 μm. (C) Enlargement of layer III non-pyramidal cell. Bar=100 μm. (D) Dendritic spines on dendrite of non pyramidal cell of pn21 α1 KO mice. Bar=20 μm.

primer: CCAGGGCAGGAATCAG AGTCT), the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH; gi 20984453 (nt 30–116), which was used for normalization of the expression levels for the total RNA input. Amplicons were chosen within a range of 80–120 bp. Each primer-combination was tested on amplification efficiency and only those that had a value between 1.8 and 2 were accepted. Copy DNA (cDNA) quantification was performed on Perkin-Elmer ABI PRISM 7700 sequence de-

Pyramidal cells

Non Pyramidal cells
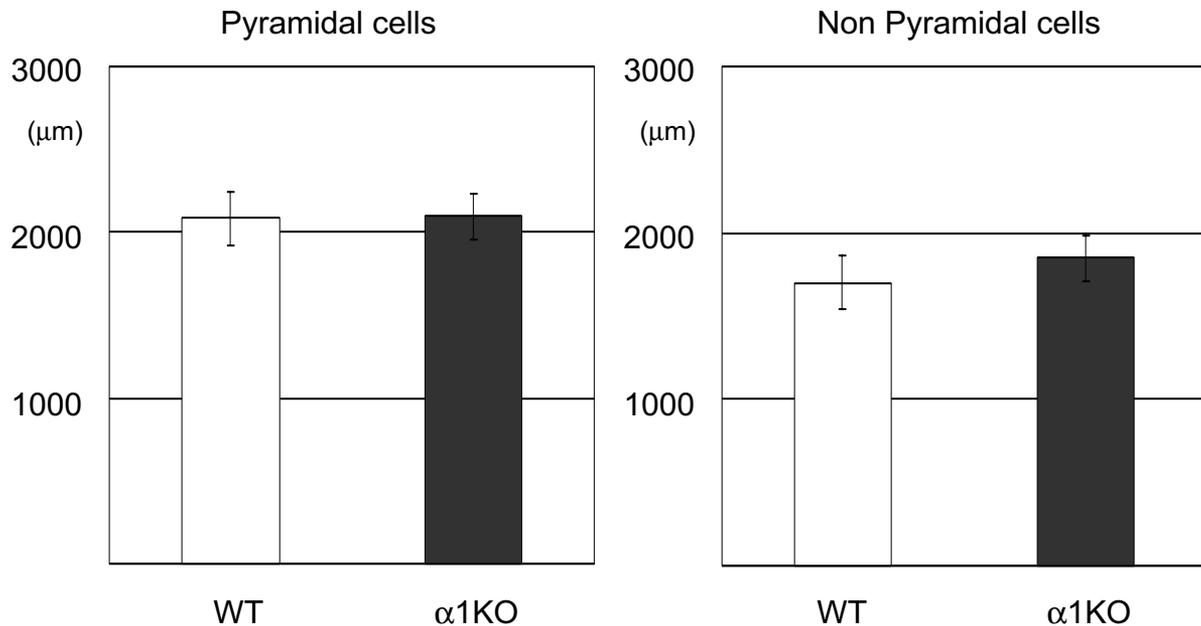
Fig. 2. Dendritic arborization. Total dendritic length was measured in pyramidal cells and nonpyramidal cells in WT and α1 KO mice. Shown are the means with S.E.M. No significant change was observed (pyramidal cells: $n=17$, WT, three mice; $n=15$, α1 KO, three mice; non pyramidal cells: $n=12$, WT, three mice; $n=8$, α1 KO, three mice).

tection system (PE Applied) using 45 cycles (95 °C, 15 s; 59 °C, 1 min) on 0.3 μl cDNA per 20 μl reaction (SYBR green 2×mix; PE Applied). Regulation of transcription in the α1 KO mice compared with the WT mice was calculated by: regulation= $E^{([Ct\ geneX\ WT-\ Ct\ GAPDH\ WT]-[Ct\ geneX\ \alpha1KO-Ct\ GAPDH\ \alpha1KO])}$, where Ct (geneX, GAPDH)=# cycles at which the PCR product reaches the set threshold value (0.03×ΔRn; ΔRn=relative amount of fluorescent PCR product compared with internal reference dye) and E=the amplification efficiency, which was considered 2 for all primers. Significant regulation between the α1 KO and the WT group was examined by using an unpaired t-test on the GAPDH subtracted data.

RESULTS

Similar dendritic arborization in α1 KO and WT mice

Analysis of the dendritic morphology revealed no significant differences between adult α1 KO and WT mice in total dendritic length of pyramidal or non-pyramidal cells (Fig. 2), nor in intermediate or terminal segment lengths (Table 1). This indicated that overall dendritic outgrowth was not changed. In addition, no differences were observed in the number of dendritic segments per neuron or the number of tips per dendrite (Table 1). Thus, the degree of branching of the dendrites was not affected either.

Spine density in α1 KO mice compared with WT mice

In order to investigate spine maturation, we quantified spine densities on the first intersegment length of pyramidal dendrites (Fig. 3A) in α1 KO and WT mice at different stages of postnatal visual cortex development. This was done at pn14 (when α1 upregulation has just started in WT mice), at pn21 (when α1 upregulation reaches a plateau level) and at the adult stage.

In the WT mice total spine density doubled during the third postnatal week and continued to increase thereafter (Fig. 3B). Total spine density was increased in the α1 KO mice at pn14 when compared with WT mice (Fig. 3B). However, it hardly increased thereafter. At pn21, total spine density was equal to that seen in the WT, whereas in adult mice it was significantly decreased.

Since basal and apical dendrites develop differently (Baker et al., 1997; McAllister et al., 1995) we also discriminated between spines that were located on basal dendrites and those on apical dendrites. This showed that the increased spine density in α1 KO mice at pn14 is mainly due to a change in basal dendritic spine number (Fig. 3B, right graph). In contrast, the decreased spine density in the adult mice was due to a decrease in apical dendritic spine number.

Spine maturation in α1 KO mice compared with WT mice

Spines appear in diverse morphologies, especially during development (Harris, 1999). We have, therefore, discriminated between filiform-shaped protrusions (filopodia) and mushroom-shaped spines (see Fig. 3A and Experimental Procedures section for selection criteria). At pn14 filopodia were present in WT mice, but they decreased in number during the following week to completely disappear at the adult age (Fig. 3C). In contrast, the filopodia density was relatively high in the α1 KO mice at pn14 compared with WT mice (3.2 per 10 μm (α1 KO) versus 1.4 per 10 μm (WT; $P<0.05$). This difference was almost exclusively due to an increase in filopodia density on basal dendrites (Fig. 3C, right graph). During the following week, filopodia density also decreased in the α1KO mice, but at a reduced

Table 1. Total, intermediate and terminal segment length, number of dendritic segments and number of tips per dendrite of basal and apical dendrites in pyramidal cells and in non-pyramidal cells are presented

	WT	α1 KO
Total length (μm)		
Pyramidal (basal)	1278±122	1275±120
	n=17	n=15
Pyramidal (apical)	806±74	820±62
	n=17	n=15
Non-pyramidal	1699±162	1850±135
	n=12	n=8
Intermediate segment length (μm)		
Pyramidal (basal)	16.5±0.9	17.5±0.9
	n=17	n=15
Pyramidal (apical)	25.6±1.8	28.9±3.0
	n=17	n=15
Non-pyramidal	23.6±3.5	20.9±2.8
	n=12	n=8
Terminal segment length (μm)		
Pyramidal (basal)	54.8±4.6	56.9±3.3
	n=17	n=15
Pyramidal (apical)	54.7±2.7	53.9±3.2
	n=17	n=15
Non-pyramidal	59.6±4.7	59.6±3.7
	n=12	n=8
# dendritic segments		
Pyramidal (basal)	13.9±1.3	13.6±1.8
	n=17	n=15
Pyramidal (apical)	9.5±1.0	9.5±0.8
	n=17	n=15
Non-pyramidal	16.9±2.1	19.4±2.2
	n=12	n=8
# tips per dendrite		
Pyramidal (basal)	19.5±1.5	18.9±1.8
	n=17	n=15
Pyramidal (apical)	10.5±1.0	10.5±0.8
	n=17	n=15
Non-pyramidal	23.3±2.5	25.1±2.3
	n=12	n=8

Data represent means±SEM (n=number).

rate, compared with the WT mice (2 per 10 μm [α1KO] versus 0.8 per 10 μm [WT]; $P<0.05$). The increased filopodia density at that age compared with the WT mice was visible both at basal and apical dendrites (Fig. 3C, right graph). Even in the adult α1 KO mice, filopodia were still present both on apical and on basal dendrites, whereas these had virtually disappeared in the WT mice (0.8 per 10 μm [α1KO] versus 0.1 per 10 μm [WT]; $P<0.05$).

We observed a steady increase in mushroom-shaped spines in the WT mice (Fig. 3D; $P<0.05$): a doubling in mushroom spine density was seen between pn14 and pn21 (2.5 per 10 μm [pn14] versus 6.7 per 10 μm [pn21]; $P<0.05$). After pn21 mushroom spine density still increased to a density of 9.1 per 10 μm in the adult mice. Although the α1 KO mice display an equal density of mushroom spines at pn14, compared with the WT mice, their density is significantly lower at later ages, both at pn21 (5.1 per 10 μm [α1 KO] versus 6.7 per 10 μm [WT]; $P<0.05$) and in the adults (6.2 per 10 μm [α1 KO] versus 9.1 per 10 μm [WT]; $P<0.05$). This difference was ob-
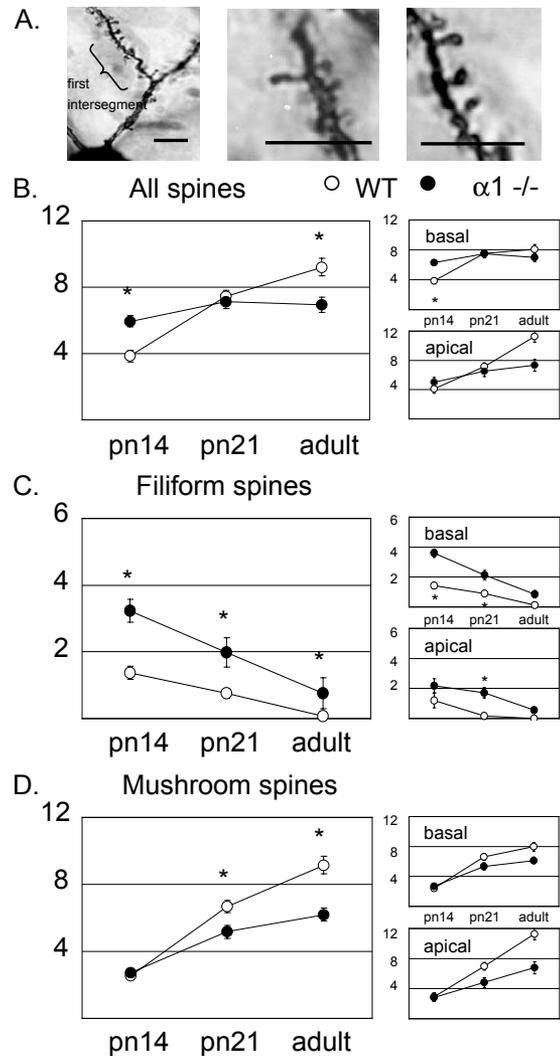
Fig. 3. Developmental changes in spine densities. (A) Dendritic spines were counted along 10 μm on the first intersegment length from the cell soma (left, bar=2 μm) and were subdivided in filopodia (middle, bar=1 μm) and mushroom-shaped spines (right, bar=1 μm). (B left graph) Number of spines per 10 μm in α1 KO mice are increased at pn14 ($P<0.01$; n=43, WT, four mice; n=52, α1 KO, four mice), equal at pn21 (n=58, WT; n=31, α1 KO, four mice) and decreased in the adult compared with WT mice ($P<0.01$; n=42, WT, three mice; n=50, α1 KO, three mice). (B right graphs) At pn14 the increased spine density is due to an increase in basal dendrites ($P<0.05$). In the adult the decreased spine density is noticeably observed on the apical dendrites (ns.; basal dendrites pn14: n=33, WT, four mice; n=39, α1 KO, four mice; pn21: n=46, WT, four mice; n=31, α1 KO, four mice; adult: n=27, WT, three mice; n=36, α1 KO, three mice; apical dendrites pn14: n=10, WT, four mice; n=13, α1 KO, four mice; pn21: n=12, WT, four mice; n=17, α1 KO, four mice; adult: n=15, WT, three mice; n=14, α1 KO, three mice). (C left graph) Number of filiform-shaped spines in the α1 KO mice were significantly increased both at pn14 ($P<0.05$) at pn21 ($P<0.05$) and in the adult mice ($P<0.05$; C right graphs). Filopodia density on basal dendrites is increased at pn14 and pn21 ($P<0.05$). On apical dendrites filopodia density is increased at pn21 ($P<0.05$). (D left graph) Number of mushroom-shaped spines in the α1 KO mice is equal at pn14 and significantly decreased both at pn21 ($P<0.05$) and in the adult animals ($P<0.05$). (D right graphs) Basal and apical dendritic mushroom spine density is decreased at pn21 and in the adult mice, however not significantly.

Table 2. Quantitative analysis of the different GABA$_A$ α subunits, BDNF and PSD-95 mRNA levels in L.I-IV visual cortex by means of quantitative PCR in WT and α1 KO mice (n=4, in duplo)

	WT	α1 KO
α1	0.133±0.008	0.0006±0.0002**
α2	0.033±0.003	0.032±0.002
α3	0.033±0.002	0.032±0.002
α4	0.017±0.001	0.014±0.001
α5	0.016±0.002	0.029±0.005
BDNF	0.009±0.001	0.013±0.002
PSD-95	0.456±0.010	0.392±0.015*

Values are corrected for GAPDH expression and calculated as described in Experimental Procedures. Asterisks indicate significant difference between WT and α1 KO expression (Student's t-test, * $P<0.05$; ** $P<0.01$).

served both on basal and apical dendrites, though more profound on the apical dendrites (Fig. 3D, right panel).

Thus, initially at pn14, we observe an increased outgrowth of juvenile filopodia in α1 KO mice, mainly on basal dendrites. At later stages however, the spine maturation at basal but in particular at apical dendritic spines is disturbed. Whether the initial difference in the rate of development of spine density in basal versus apical dendrite is of physiological significance is currently unclear.

GABA$_A$ α-subunits and BDNF expression in α1 KO mice

Altered spine maturation can be a direct or indirect consequence of the deletion of the α1 subunit in the α1 KO mice or, alternatively, be caused by compensatory regulation of other α subunits. We measured mRNA levels of the other α subunits in the α1 KO mice and found that they were not significantly changed compared with the levels in the WT mice (Table 2). Spine maturation can be directly influenced by the growth factor BDNF (Horch et al., 1999). To test whether α1 deletion had indirectly affected BDNF expression, BDNF levels were investigated. No significant changes were observed (Table 2). In line with the observed decrease in spine density however, we measured a small but significant downregulation in the postsynaptic density protein, associated with excitatory synapses, PSD-95 (Table 2; Marrs et al., 2001).

DISCUSSION

Many of the processes that are involved in the construction of the cortical network during a critical period in development (Katz and Shatz, 1996) are sculpted by neuronal activity. For instance, visual deprivation studies have shown that axonal growth and dendritic arborization are decreased in the absence of normal sensory input (Coleman and Riesen, 1968; Friedlander et al., 1982; Wiesel and Hubel, 1965). Moreover, in vivo, N-methyl-D-aspartate (NMDA) receptor blockade decreases dendritic growth and branching (Vogel and Prittie, 1995). In addition, the formation and maturation of dendritic spines (Harris et al., 1995) appears also to be modulated by activation of α-ami-

no-3-hydroxy-methyl-4-isoaxoleproprionate (AMPA) and NMDA receptors (Yuste and Bonhoeffer, 2001; Das et al., 1998; Kalb, 1994; Rajan et al., 1999).

Most studies so far have focused on the role of glutamatergic receptors as the site of modulation of dendritic outgrowth and spine formation by neuronal activity. However, GABAergic inhibition profoundly modulates glutamatergic activity by exerting control over neuronal firing and synchronization (Krnjevic, 1997; Tamas et al., 2000). Recent studies indicate that the GABAergic system may play a prominent role in regulating the onset of the critical period, during which the activity dependent outgrowth processes take place (Fagiolini and Hensch, 2000; Hensch et al., 1998).

In this study, we observed a steady increase in total spine density in the visual cortex of WT mice during early postnatal development. This profound increase between pn14 and pn21 is likely to be induced by increased levels of activity following the moment of eye opening around pn13 (Shatz, 1996). At pn14 a significant proportion of the spines (ca. 25%) had a filopodia-like shape. Imaging studies have suggested that filopodia may act as spine precursors (Harris et al., 1995) that first protrude and then retract, after which mature mushroom-shaped spines evolve (Dailey and Smith, 1996; Fiala et al., 1998; Jontes and Smith, 2000; Ziv and Smith, 1996). Although this question has not been resolved yet (Bonhoeffer and Yuste, 2002), it is noteworthy that in the WT mice, the density of filopodia indeed gradually disappeared, while the density of mushroom-shaped spines significantly increased during the same period of development.

In juvenile α1 KO mice (pn14) the overall density of spines appeared to be increased. However, this was mainly due to an increased proportion of filopodia. At pn21 the ratio of filiform- versus mushroom-shaped spines was still increased in α1 KO, compared with WT. It is unclear at this point how this may come about. Analysis of miniature inhibitory postsynaptic currents (IPSCs) in the α1 KO mice revealed an increased efficacy of the synaptic GABA$_A$ receptor (Bosman et al., 2002) due to the increased synaptic current decay time and an additional increase in the amplitude monoquantal synaptic currents. Moreover the frequency of mIPSCs was not affected, which implies that there was no reduction in number of synapses impinging per cell. Thus during neonatal development an enhanced inhibitory tone may have occurred within the cortical network in α1 KO mice which may suppress overall neuronal activity. This is confirmed by the observation that 40 Hz γ-band frequency oscillations are reduced in visual cortex slices of α1 KO mice (L. Bosman and H. Lodder, personal communication; unpublished data). It would be premature to conclude that there exists a causal relation between an increased inhibitory tone and an increased density of filopodia. However, in line with this hypothesis, several studies have shown that a decrease in neuronal network activity, for instance due to application of tetrodotoxin or 2-amino-5-phosphonovalerate, may lead to an increase in spine formation on immature dendrites (Dalva et al., 1994; Rocha and Sur, 1995).

At pn21 and in adult, we observed a decrease in mature mushroom spine density in α1 KO mice compared

with WT mice. In line with this observation, we report a slight downregulation of PSD-95, a postsynaptic density protein that is associated with mature glutamatergic synapses (Marrs et al., 2001). If filopodia are the precursor stage of mushroom-shaped spines (but Bonhoeffer and Yuste, 2002 for review), it is possible that in α1 KO this transition process was impaired. In addition to a failure in filopodia-to-mushroom-spine transition, our observation could also be explained by impaired consolidation of mushroom-shaped spines, which would subsequently reverse to the filiform state. Hence 'online' functional imaging experiments (Yuste and Bonhoeffer, 2001) should be performed to test which stage of spine maturation is exactly affected in α1 KO mice.

Long-term potentiation (LTP) is thought to play a key role in synapse formation and consolidation and possibly also in spine maturation (Isaac et al., 1997; Kirkwood and Bear, 1994; Yuste and Bonhoeffer, 2001). GABAergic transmission can interfere with the induction of LTP (Higashima et al., 1998; Tomasulo et al., 1993), by shunting AMPA receptor-mediated synaptic currents and thereby reducing the extent of voltage dependent NMDA receptor activation. Possibly via this mechanism, the change in GABAergic synaptic efficacy may have led to the impairment in spine maturation in the α1 KO mice.

However, also other explanations should be considered. For instance steroid levels are known to affect spine maturation and density (McEwen et al., 2001). Therefore, overall changes in hormonal regulation due to altered GABAergic transmission in other brain areas might also play a role.

The implications of the differences we observed in spine densities between apical and basal dendrites remain unclear. Two-photon microscopy may elucidate whether differential subcellular expression of different types of GABA$_A$ receptors occurs. Furthermore, the fact that no alterations were observed in dendritic arborization suggests that the process of dendritic outgrowth is less sensitive to modulation of neuronal activity, or occurs at an earlier developmental stage.

We conclude that while the density of filopodia was enhanced at an early age in α1 KO mice, formation of mature mushroom-shaped spines was impaired, possibly due to the fact that synaptic contacts are not properly established and/or consolidated. As a result, the overall spine density is decreased in the adult α1 KO mice. The data presented here indicate that, in addition to other factors, proper maturation of the GABA$_A$ receptor (Bosman et al., 2002) at the level of individual synapses, may affect the process of spine formation. In addition to our findings, it is noteworthy that not only an enhanced GABAergic inhibition (this study) but also a decreased impact of neocortical GABA input, as is observed in glutamic acid decarboxylase 65 (GAD65) KO mice, interferes with neocortical development. These GAD65 KO mice lack a proper onset of the critical period in visual cortex *in vivo* (Fagiolini and Hensch, 2000; Hensch et al., 1998). Thus, maturation of the GABAergic system, both in terms of number of synapses and in terms of receptor functioning, appears to be crucial for experience-dependent fine tuning of cortical connections during the critical period.

REFERENCES

Araki T, Kiyama H, Tohyama M (1992) GABA$_A$ receptor subunit messenger RNAs show differential expression during cortical development in the rat brain. Neuroscience 51:583–591.

Baker RE, Wolters P, Van Pelt J (1997) Chronic blockade of glutamate-mediated bioelectric activity in long-term organotypic neocortical explants differentially effects pyramidal/non-pyramidal dendritic morphology. Brain Res Dev Brain Res 104:31–39.

Bonhoeffer T, Yuste R (2002) Spine motility: phenomenology, mechanisms, and function. Neuron 35:1019–1027.

Bosman LW, Rosahl TW, Brussaard AB (2002) Neonatal development of the rat visual cortex: synaptic function of GABA$_A$ receptor α subunits. J Physiol 545:169–181.

Coleman PD, Riesen AH (1968) Environmental effects on cortical dendritic fields: I. Rearing in the dark. J Anat 102:363–374.

Dailey ME, Smith SJ (1996) The dynamics of dendritic structure in developing hippocampal slices. J Neurosci 16:2983–2994.

Dalva MB, Ghosh A, Shatz CJ (1994) Independent control of dendritic and axonal form in the developing lateral geniculate nucleus. J Neurosci 14:3588–3602.

Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 393:377–381.

Drakew A, Frotscher M, Heimrich B (1999) Blockade of neuronal activity alters spine maturation of dentate granule cells but not their dendritic arborization. Neuroscience 94:767–774.

Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183–186.

Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci 18:8900–8911.

Friedlander MJ, Stanford LR, Sherman SM (1982) Effects of monocular deprivation on the structure-function relationship of individual neurons in the cat's lateral geniculate nucleus. J Neurosci 2:321–330.

Fritschy JM, Paysan J, Enna A, Mohler H (1994) Switch in the expression of rat GABA$_A$-receptor subtypes during postnatal development: an immunohistochemical study. J Neurosci 14:5302–5324.

Harris BT, Costa E, Grayson DR (1995) Exposure of neuronal cultures to K$^+$ depolarization or to N-methyl-D-aspartate increases the transcription of genes encoding the α1 and α5 GABA$_A$ receptor subunits. Brain Res Mol Brain Res 28:338–342.

Harris KM (1999) Structure, development, and plasticity of dendritic spines. Curr Opin Neurobiol 9:343–348.

Harris KM, Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 17:341–371.

Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282:1504–1508.

Higashima M, Kinoshita H, Koshino Y (1998) Differences in the effects of zolpidem and diazepam on recurrent inhibition and long-term potentiation in rat hippocampal slices. Neurosci Lett 245:77–80.

Horch HW, Kruttgen A, Portbury SD, Katz LC (1999) Destabilization of cortical dendrites and spines by BDNF. Neuron 23:353–364.

Isaac JT, Crair MC, Nicoll RA, Malenka RC (1997) Silent synapses during development of thalamocortical inputs. Neuron 18:269–280.

Jontes JD, Smith SJ (2000) Filopodia, spines, and the generation of synaptic diversity. Neuron 27:11–14.

Kalb RG (1994) Regulation of motor neuron dendrite growth by NMDA receptor activation. Development 120:3063–3071.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133–1138.

Kirkwood A, Bear MF (1994) Hebbian synapses in visual cortex. J Neurosci 14:1634–1645.

Kossel AH, Williams CV, Schweizer M, Kater SB (1997) Afferent innervation influences the development of dendritic branches and spines via both activity-dependent and non-activity-dependent mechanisms. J Neurosci 17:6314–6324.

Krnjevic K (1997) Role of GABA in cerebral cortex. Can J Physiol Pharmacol 75:439–451.

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen GABA$_A$ receptor subunit mRNAs in the rat brain: III. Embryonic and postnatal development. J Neurosci 12:4151–4172.

Marrs GS, Green SH, Dailey ME (2001) Rapid formation and remodeling of postsynaptic densities in developing dendrites. Nat Neurosci 4:1006–1013.

McAllister AK, Lo DC, Katz LC (1995) Neurotrophins regulate dendritic growth in developing visual cortex. Neuron 15:791–803.

McEwen B, Akama K, Alves S, Brake WG, Bulloch K, Lee S, Li C, Yuen G, Milner TA (2001) Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. Proc Natl Acad Sci USA 98:7093–7100.

McKinney RA, Capogna M, Durr R, Gahwiler BH, Thompson SM (1999) Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat Neurosci 2:44–49.

Overdijk J, Uylings HB, Kuypers K, Kamstra W (1978) An economical, semi-automatic system for measuring cellular tree structures in three dimensions, with special emphasis on Golgi-impregnated neurons. J Microsc 114:271–284.

Rajan I, Witte S, Cline HT (1999) NMDA receptor activity stabilizes presynaptic retinotectal axons and postsynaptic optic tectal cell dendrites in vivo. J Neurobiol 38:357–368.

Rocha M, Sur M (1995) Rapid acquisition of dendritic spines by visual thalamic neurons after blockade of N-methyl-D-aspartate receptors. Proc Natl Acad Sci USA 92:8026–8030.

Shatz CJ (1996) Emergence of order in visual system development. Proc Natl Acad Sci USA 93:602–608.

Sur C, Wafford KA, Reynolds DS, Hadingham KL, Bromidge F, Macaulay A, Collinson N, O'Meara G, Howell O, Newman R, et al (2001) Loss of the major GABA$_A$ receptor subtype in the brain is not lethal in mice. J Neurosci 21:3409–3418.

Tamas G, Buhl EH, Lorincz A, Somogyi P (2000) Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nat Neurosci 3:366–371.

Tomasulo RA, Ramirez JJ, Steward O (1993) Synaptic inhibition regulates associative interactions between afferents during the induction of long-term potentiation and depression. Proc Natl Acad Sci USA 90:11578–11582.

Uylings HB, Ruiz-Marcos A, van Pelt J (1986) The metric analysis of three-dimensional dendritic tree patterns: a methodological review. J Neurosci Methods 18:127–151.

Vicini S, Ferguson C, Prybylowski K, Kralic J, Morrow AL, Homanics GE (2001) GABA$_A$ receptor α1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. J Neurosci 21:3009–3016.

Vogel MW, Prittie J (1995) Purkinje cell dendritic arbors in chick embryos following chronic treatment with an N-methyl-D-aspartate receptor antagonist. J Neurobiol 26:537–552.

Wiesel TN, Hubel DH (1965) Extent of recovery from the effects of visual deprivation in kittens. J Neurophysiol 28:1060–1072.

Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071–1089.

Ziv NE, Smith SJ (1996) Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron 17:91–102.