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Postnatal alterations of the inhibitory synaptic responses recorded from cortical pyramidal neurons in the *Lis1/sLis1* mutant mouse

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Mutations in the mouse *Lis1* gene produce severe alterations in the developing cortex. We have examined some electrophysiological responses of cortical pyramidal neurons during the early postnatal development of *Lis/sLis1* mutant mice. In P7 and P30 *Lis1/sLis1* neurons we detected a lower frequency and slower decay phase of mIPSCs, and at P30 the mIPSCs amplitude and the action potential duration were reduced. Zolpidem (an agonist of $GABA_A$ receptors containing the α_1 subunit) neither modified the amplitude nor the decay time of mIPSCs at P7 in *Lis1/sLis1* neurons, whereas it increased the decay time at P30. The levels of $GABA_A$ receptor α_1 subunit mRNA were reduced in the *Lis1/sLis1* brain at P7 and P30, whereas reduced levels of the corresponding protein were only found at P7. These results demonstrate the presence of functional alterations in the postnatal *Lis1/sLis1* cortex and point to abnormalities in $GABA_A$ receptor subunit switching processes during postnatal development.

Introduction

Abnormalities in the migration of neurons into the embryonic cortex lead in extreme cases to loss of normal convolutions of the cortex in humans, known as lissencephaly ("smooth brain") (Aicardi, 1989; Barth, 1987; reviewed by Reiner and Coquelle, 2005). Lissencephaly is the predominant characteristic of a set of diseases, which are distinguished also by severe mental retardation, epilepsy, recurrent seizures, and motor impairment. Mutations in *LIS1*, an autosomal gene located on chromosome 17p13.3, are known to result in lissencephaly (Reiner et al., 1993). Since the mutations occur only in a single allele, the disease mechanism has been considered as haploinsufficiency.

LIS1 was identified as the β subunit of the platelet-activating factor acetylhydrolase (PAF-AH) isoform Ib (Hattori et al., 1994) which is the enzyme that inactivates platelet-activating factor (PAF). LIS1 is also a protein that interacts with tubulin and microtubules (Sapir et al., 1999) which directly influences microtubule dynamics during neuronal differentiation and migration. In addition, LIS1 interacts with the molecular motor cytoplasmic dynein and regulates its activity (Faulkner et al., 2000; Smith et al., 2000) and it has been shown that dynein is capable of forming a complex with dynactin subunits with which LIS1 can also interact (Tai et al., 2002).

Reduction of LIS1 levels and the introduction of mutations to the mouse *Lis1* gene have proven to be extremely useful in dissecting the complex roles of LIS1 during brain development (Cahana et al., 2001; Hirotsune et al., 1998; Shu et al., 2004; Tsai et al., 2005). This goal has been achieved by the generation of transgenic mice through homologous gene targeting (Cahana et al., 2001; Hirotsune et al., 1998), or more recently, by introduction of siRNA using *in utero* electroporation (Shu et al., 2004; Tsai et al., 2005). Early embryonic lethality was observed in homozygous mutant *Lis1* mouse embryos which die following implantation (Cahana et al., 2001; Cahana et al., 2003; Hirotsune et al., 1998). Furthermore, as seen in heterozygote and compound heterozygote animals it is clear that there is a dose-specific response to reduction in LIS1 protein levels. This dosage sensitivity of LIS1 is probably due to its central role in multiple and important protein complexes (reviewed by Reiner, 2000; Reiner et al., 2006). Half dosage of LIS1 only slightly affected neuronal migration both in the cortex and in the hippocampus and the organization of the adult cortical layers appeared normal. However, further reduction in LIS1 levels which occurs in the compound heterozygote severely obstructed both cortical and hippocampal organization (Fleck et al., 2000; Gambello et al., 2003; Hirotsune et al., 1998). Within the hippocampus of Lis1−/+ mice, abnormal structures of both pyramidal and dentate gyrus neurons were observed as well as improper positioning of neurons (Fleck et al., 2000), together with abnormal electrophysiological features, such as hyperexcitability at Schaffer collateral–CA1 synapses and depression of mossy fiber–CA3 transmission. In addition, the dynamic range of frequency-dependent facilitation of

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Lis1−/+ mossy fiber transmission was less than that measured in WT brains. Consequently, *Lis1*−/+ hippocampi are prone to electrographic seizure activity and serve as a model of epilepsy (Fleck et al., 2000). Furthermore, many of the heterozygous mice die from epileptic seizures (Hirotsune et al., 1998).

In addition to brain organization, motor activity, learning, and memory have also been reported to be altered in Lis1−/+ mutant mice (Paylor et al., 1999). Other alterations include radial neuronal migration and the tangential migration of inhibitory interneurons is affected as well (McManus et al., 2004). Interneuron abnormalities may be relevant to the disease phenotype since they were also observed in patients with Miller–Dieker syndrome (Pancoast et al., 2005). Interestingly, a worm LIS1 mutant exhibited convulsions mimicking epilepsy when treated with a chemical antagonist of gamma-aminobutyric acid (GABA) neurotransmitter signaling (Williams et al., 2004). Therefore, future functional studies related to neuronal activity during postnatal development may enhance our understanding of the disease phenotype.

The phenotype of mice with a hypomorphic *Lis1* allele, referred to as *Lis1/sLis1* (Cahana et al., 2001; reviewed by Reiner et al., 2002), somewhat differs from that of the heterozygous Lis1−/+ mice (Hirotsune et al., 1998). In *Lis1/sLis1* mice, transient inhibition of neuronal migration, abnormal morphology of radial glia and cortical pyramidal neurons, and a delay in the thalamo-cortical innervation were observed. However, the gross structure of the hippocampus was normal, and furthermore these mice did not suffer from epileptic seizures. In contrast, 5% of *Lis1*−/+ mutant mice die from seizures at 3–5 weeks of age (Hirotsune et al., 1998). The *Lis1* mutation generated in the *Lis1/sLis1* mice resembles that of a patient with an in-frame N-terminal deletion (Fogli et al., 1999). The mutant protein was found to be incapable of dimerization (Cahana et al., 2001) due to the N-terminal deletion. The deletion encompassed the conserved Lis-H domain (Emes and Ponting, 2001), which is crucial for LIS1 dimerization (Gerlitz et al., 2005; Kim et al., 2004; Tarricone et al., 2004). Analysis in the mutant mice revealed partial protein synthesis from the mutated allele, and a similar partial protein was detected in this particular patient (Fogli et al., 1999). In comparison with patients with classical lissencephaly (Dobyns et al., 1993) this patient showed a milder degree of cortical abnormality, with diffuse pachygyria and small areas of agyria in the posterior convexity. The patient also had mild hypotonia, was able to walk unassisted (at 3 years 4 months), had no epilepsy, was cognitively less impaired, and was able to respond to simple orders.

In the light of the relevance of the *Lis1/sLis1* mouse model to the lissencephaly phenotype, we initiated a study aimed to characterize the postnatal electrophysiological properties of pyramidal cortical neurons, which throws fresh light on our understanding of the functional status of the neocortex in the *Lis1/sLis1* mouse. Preliminary results were presented at the 2003 Annual Meeting of the Society for Neuroscience (Valdés-Sánchez et al., 2003).

Results

Electrophysiological responses of Lis1/sLis1 pyramidal neurons

The electrophysiological responses to intracellular current pulses of pyramidal neurons recorded from superficial layers of *Lis1/sLis1* cortical slices at postnatal ages P7 and P30 are shown in Fig. 1 and Table 1. Significantly, the duration of the action

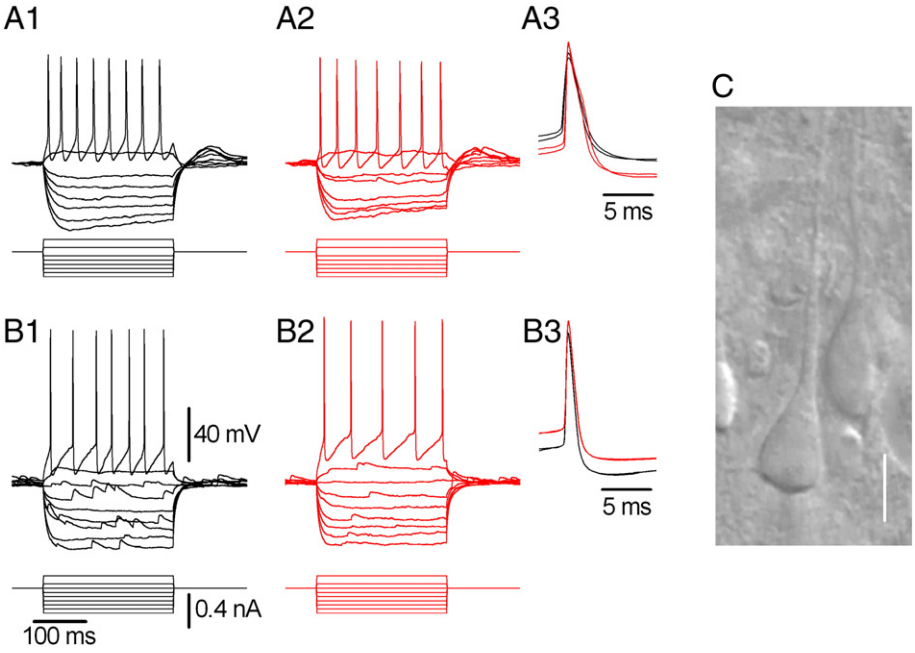

Fig. 1. Electrophysiological responses of pyramidal neurons in WT and Lis1/sLis1 cortical slices. Membrane potential responses and firing patterns recorded in response to hyper- and depolarizing current pulses from P7 pyramidal neurons (A1 WT and A2 *Lis1/sLis1*) and P30 neurons (B1 WT and B2 *Lis1/sLis1*). The resting membrane potential of these neurons was −63 mV (A1), −70 mV (A2), −65 mV (B1), and −68 mV (B2). Scale bars in panel B1 apply to panels A1, A2, and B2. Panels A3 and B3, action potentials shown at an expanded time scale; two superimposed consecutive action potentials from WT and *Lis1/sLis1* neurons (black and red traces respectively) from P7 (A3) and P30 (B3) animals. The action potentials in panels A3 and B3 were taken from the recordings in A1/A2 and B1/B2, respectively; the vertical voltage scale is the same as in panel B1. (C) Pyramidal neuron from a P30 WT slice as seen under IR-DIC microscopy; scale bar, 20 μm.

Table 1
Electrophysiological parameters of pyramidal neurons recorded from WT and Lis1/sLis1 animals

	P7		P30	
	WT	*Lis1/sLis1*	WT	*Lis1/sLis1*
Resting potential (mV)	−60.6±2.5 (−52 −70) *n*=44	−66.4±2.9 (−52 −69) *n*=29	−65.4±2.4 (−54 −88) *n*=39	−66.1±3.5 (−64 −75) *n*=27
Input resistance (MΩ)	467±50.1 (88–1900) *n*=44	614±73.9 (191–1700) *n*=29	156±12.1 (47–399) *n*=39	165±17.9 (52–526) *n*=27
a.p. amplitude (mV)	66.2±1.7 (54.7–81.2) *n*=23	68.7±2.3 (55.4–78.9) *n*=11	78.4±1.5 (54.4–89.2) *n*=25	80.0±1.9 (67.1–99.7) *n*=22
a.p. duration (ms)	1.77±0.87 (1.22–2.38) *n*=23	1.66±0.13 (0.99–2.33) *n*=11	1.02±0.06 (0.57–2.14) *n*=25	0.86±0.04 * (0.47–1.15) *n*=22
Ahp amplitude (mV)	17.0±1.27 (6.2–29.3) *n*=23	15.5±1.33 (7.35–22.1) *n*=11	12.6±1.70 (4.6–23.3) *n*=25	15.4±1.78 (8.5–22.8) *n*=22

Action potential (a.p.) amplitude was measured with respect to the threshold potential, and action potential duration was measured at half amplitude. Input resistance was measured from the voltage deflection produced by small hyperpolarizing current pulses (e.g. Fig. 1) and the amplitude of the after-hyperpolarization (Ahp) was measured from the threshold level to the most negative peak. Input resistance was calculated from responses to small hyperpolarizing current pulses. The mean±s.e.m., the range (in parenthesis), and the number of observations are provided for each parameter.
* $p < 0.05$ compared with P30, WT.

potential at P30 was longer in wild type (WT) neurons versus *Lis1/sLis1* neurons (1.02 ms vs. 0.86 ms; Table 1). The firing pattern, action potential properties, membrane potential, and input resistance of *Lis1/sLis1* pyramidal neurons were similar to the "regular spiking" type described in neocortical pyramidal neurons from several species (McCormick et al., 1985; Connors and Gutnick, 1990; Kawaguchi, 1993, 1995). In addition, several changes in cortical pyramidal neuron properties which are known to occur during the first postnatal month (Picken Bahrey and Moody, 2003; Zhang, 2004) were noted in both *Lis1/sLis1* and WT neurons (Table 1). These changes consisted of decreased input resistance and action potential duration, and an increase of action potential amplitude from P7 to P30.

Miniature inhibitory postsynaptic currents

We next studied the miniature inhibitory postsynaptic currents (mIPSCs) recorded from pyramidal neurons. We observed marked differences in the frequency, amplitude, and time course of mIPSCs recorded in pyramidal neurons of WT and *Lis1/sLis1* slices prepared from P7 and P30 cortices. The mean mIPSC frequency, calculated over a period of 3 min of stable continuous recording,

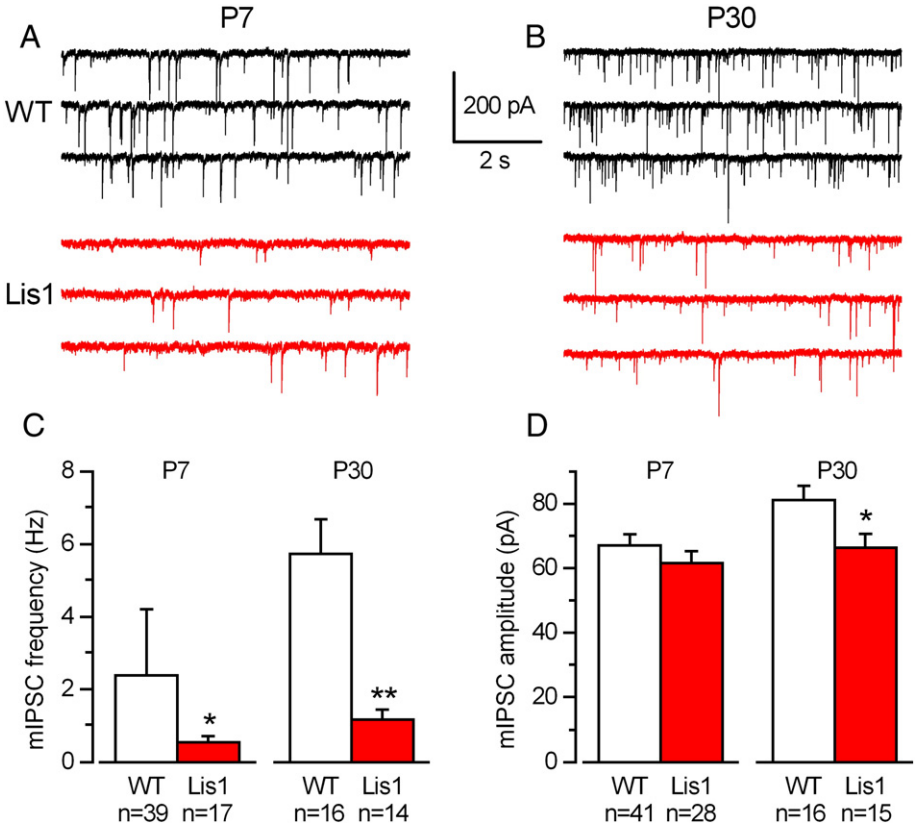

Fig. 2. Frequency and amplitude of mIPSCs recorded in pyramidal neurons from P7 and P30 animals. (A and B) Three consecutive traces of membrane currents recorded at −70 mV in the presence of TTX, CNQX, and APV in P7 (A) and P30 (B) pyramidal neurons from WT animals (black traces) and from *Lis1/sLis1* animals (red traces); scale bars apply to both panels. Frequency (C) and amplitude (D) of mIPSCs; both parameters were calculated from the average of the spontaneous currents detected (as described in Materials and methods) in a 3-minute period of continuous stable recording. *$p < 0.05$; **$p < 0.01$.

was significantly lower in *Lis1/sLis1* than in WT pyramidal neurons, at both P7 and P30 postnatal ages (Figs. 2A–C). In both WT and *Lis1/sLis1* animals, the frequency of mIPSCs increased from P7 to P30 in pyramidal neurons (Fig. 2C), corroborating previous reports in the neocortex and the hippocampus (Dunning et al., 1999; Hollrigel and Soltesz, 1997). This increase in mIPSC frequency is a consequence of the massive synaptogenesis that takes place in the cortex during this early postnatal period. The increase of mIPSC frequency from P7 to P30 was similar in WT and *Lis1/sLis1* animals (frequency P30/P7 = 2.40 for WT neurons; frequency P30/P7 = 2.14 for *Lis1/sLis1* neurons), suggesting that the postnatal rate of inhibitory synaptic formation in the neocortex is similar in WT and *Lis1/sLis1* animals.

The mIPSCs detected over a period of 3 min of stable continuous recording were averaged to calculate their amplitude and the time course of the corresponding decay phase. The amplitude of the averaged mIPSC was similar in both WT and *Lis1/sLis1* animals at P7, but was significantly smaller in the *Lis1/sLis1* neurons at P30 (Fig. 2D). The time course of the averaged mIPSC decay phase was markedly different in *Lis1/sLis1* and WT pyramidal neurons. This decay phase, quantified by the weighted time constant calculated from the fit to the sum of 2 exponentials (see Materials and methods), was slower in *Lis1/sLis1* neurons than in WT neurons, both at the P7 and at P30 postnatal ages (Fig. 3). The mIPSC decay phase was faster at P30 than at P7 in both WT and *Lis1/sLis1* animals (Fig. 3C) probably as a consequence of the postnatal changes in subunit composition which takes place during the first postnatal weeks (see below and Discussion). However, the decay phase change rate from P7 to P30 was similar in both WT and *Lis1/sLis1* neurons (the weighted time constant of the decay phase at P30 was 0.57 times the value at P7 in both groups of animals).

Presence of the α_1 subunit in the $GABA_A$ receptors

During the first 2 or 3 weeks of postnatal development, the decay phase of the mIPSCs changes due to changes in the $GABA_A$ receptor subunit composition (Bosman et al., 2002, 2005; Dunning et al., 1999; Heinen et al., 2004). Thus, α_1 subunits, which are very rare at birth, are progressively incorporated into $GABA_A$ receptors. The incorporation of the α_1 subunit produces faster mIPSCs (Goldstein et al., 2002; Vicini et al., 2001). Therefore, the difference found in the time course of the mIPSC decay between WT and *Lis1/sLis1* neurons (Fig. 3) indicates that the postnatal process of $GABA_A$ receptor subunit change may be altered in the *Lis1/sLis1* mutant, as it happens in other cortical alterations, such as cortical malformations induced by freeze lesions in rat (Hablitz and DeFazio, 2000) or in rat models of temporal lobe epilepsy (Poulter et al., 1999). This hypothesis was first tested by studying the effects of zolpidem on the amplitude and the time course of the mIPSCs at P7 and P30. Zolpidem is a $GABA_A$ receptor agonist, which is selective, at low concentrations (100 nM), for $GABA_A$ receptors containing the α_1 subunit (Ruano et al., 1992; Renard et al., 1999; Vicini et al., 2001; Korpi et al., 2002). The application of 100 nM zolpidem increased both the half amplitude decay time and the amplitude of the mIPSCs at P7 in WT neurons, while no significant changes were noted in these mIPSC parameters in P7 *Lis1/sLis1* neurons (Fig. 4). However, at P30, 100 nM zolpidem significantly increased the half amplitude decay time in WT and *Lis1/sLis1* animals, whereas the mIPSC amplitude remained unaltered by zolpidem in *Lis1/sLis1* neurons (Fig. 4).

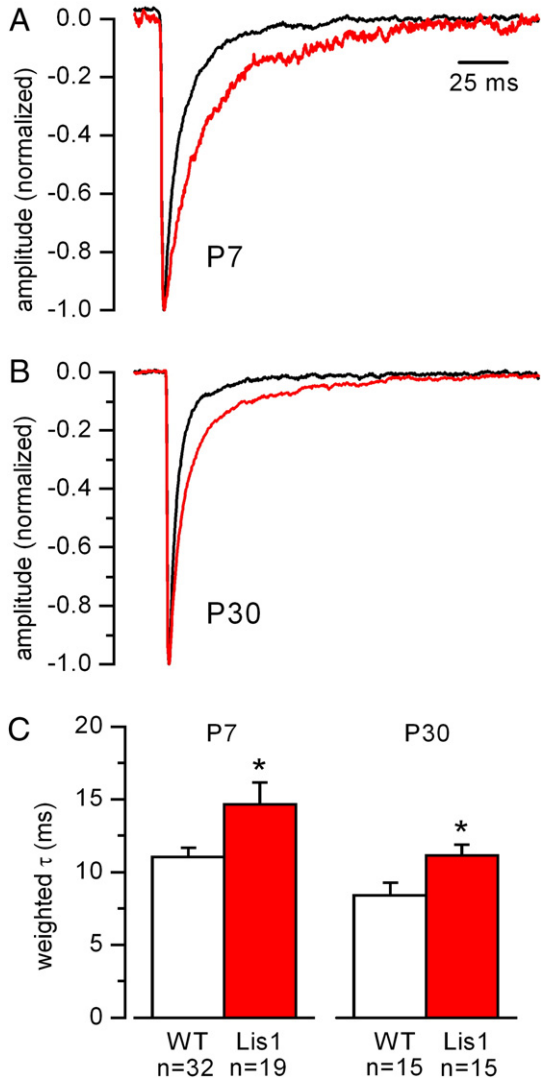

Fig. 3. Time course of the decay phase of the mIPSC recorded in pyramidal neurons from P7 and P30 animals. Superimposed traces of the averaged mIPSC recorded in pyramidal neurons from (A) P7 and (B) P30 animals (black trace WT, red trace *Lis1/sLis1*). The averages were taken from the mIPSCs detected over a 3 min period of continuous recording. The amplitude was normalized to the peak value to clearly show the differences in the decay phase; time scale bar in panel A applies to panel B. (C) Differences in the weighted time constant (calculated as described in Materials and methods) of the mIPSC decay phase in WT and *Lis1/sLis1* pyramidal neurons. $^*p < 0.05$.

Since at low concentrations zolpidem is selective for $GABA_A$ receptors containing the α_1 subunit, the above results suggest that the postnatal subunit switching process for $GABA_A$ receptors is abnormal in *Lis1/sLis1* animals. In order to assess this possibility, the levels of α_1 subunit mRNA and protein were measured using qRT-PCR and Western blot, respectively. The levels of α_1 subunit mRNA were significantly lower in *Lis1/sLis1* brain tissue at P7 and P30 (Fig. 5A). On the other hand, the levels of the protein of the α_1 $GABA_A$ receptor subunit were significantly lower in the *Lis1/sLis1* brain at P7, while they were similar at P30 (Figs. 5B, C). These results are indicative of alterations in the regulation of the α_1 subunit at both transcriptional and post-transcriptional levels in *Lis1/sLis1* animals during the first postnatal month. Thus, at early

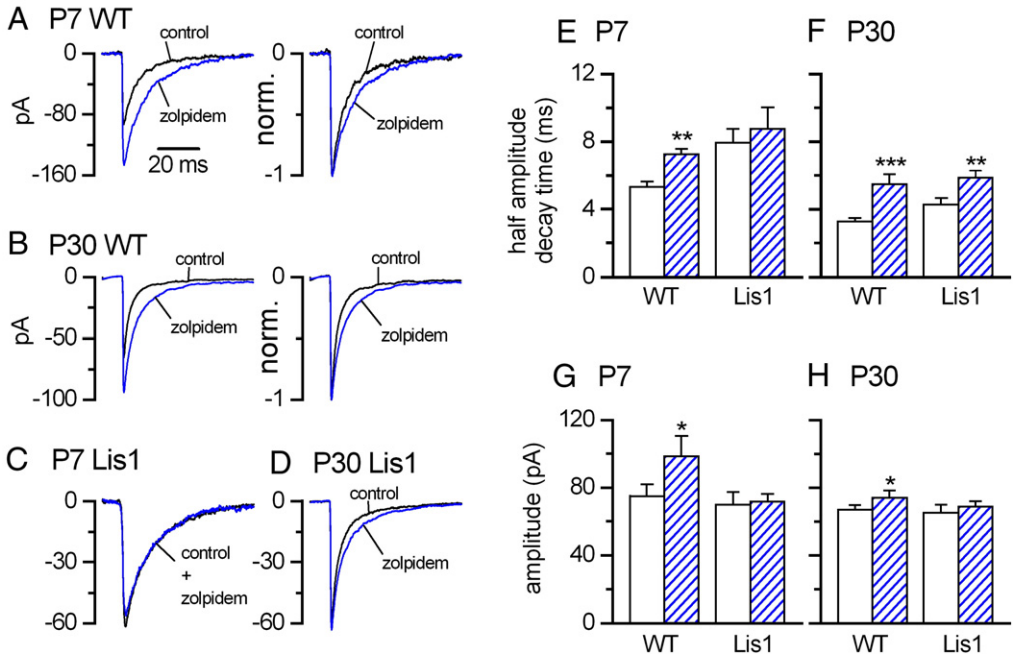

Fig. 4. Effect of 100 nM zolpidem on mIPSCs recorded in neurons from P7 animals and P30 animals. (A–D) Effects of the application of 100 nM zolpidem on the amplitude and decay time course of averaged mIPSCs recorded from a WT P7 pyramidal neuron (A), a WT P30 pyramidal neuron (B), a P7 Lis1 neuron (C), and a P30 Lis1 neuron (D). Panels A and B show the increase in mIPSC amplitude produced by zolpidem in WT neurons; the recordings on the right of panels A and B are the same recordings as on the left of each panel but normalized to peak amplitude to show clearly the difference in the decay phase time course produced by zolpidem. In P7 neurons (C and D) zolpidem did not modify the amplitude of the averaged mIPSCs. Panels A–D show the averaged mIPSCs detected over a 3-minute period of continuous recording under control conditions (black traces) and after a 3-minute application of 100 nM zolpidem (blue traces). (E–H) Plots of the mean values of half amplitude decay time and amplitude of averaged mIPSCs in control and in the presence of 100 nM zolpidem obtained from P7 neurons (E and G; WT $n=8$; *Lis1/sLis1* $n=10$) and from P30 neurons (F and H; WT $n=13$; *Lis1/sLis1* $n=18$) White bars, control; hatched bars, effect of zolpidem; the vertical axes of panels F and H are the same in panels E and G respectively. Time scale in panel A applies to panels A to D. $*p<0.05$; $**p<0.005$; $***p<0.0005$ control vs. zolpidem.

stages of postnatal development (P7) the number of $GABA_A$ receptors containing the α_1 subunit is lower in *Lis1/sLis1* animals as indicated by the lower levels of mRNA and protein and by the fact that at this age zolpidem had no effect on *Lis1/sLis1* mIPSCs (see Fig. 4). However, at a later stage of postnatal development (P30) the levels of α_1 mRNA were still decreased, but the levels of protein were normal and the number of $GABA_A$ receptors containing the α_1 subunit was closer to that found in the normal pyramidal neurons since at P30 zolpidem had a partial effect on mIPSCs (it increased the half amplitude decay time but not the amplitude; Fig. 4).

Discussion

Our findings demonstrate the presence of functional alterations in the postnatal cerebral cortex of the *Lis1/sLis1* mutant mouse: a decrease of the frequency of mIPSC recorded from pyramidal neurons at postnatal days 7 and 30 and a lower mIPSC amplitude at P30 and a slower decay phase of the mIPSC recorded from pyramidal neurons at P7 and at P30. These alterations are indicative of an abnormal function of the cortical inhibitory system in the *Lis1/sLis1* mutant mouse. Moreover, the action potential duration of pyramidal neurons was shorter in P30 *Lis1/sLis1* neurons, suggesting the presence of more extensive electrophysiological alterations in the *Lis1/sLis1* postnatal cortex. The finding at P30 of differences not present at P7 (shorter action potential duration) may indicate the presence of electrophysiological alterations which are not present at birth but appear later in the postnatal development in *Lis1/sLis1* animals. However, it is currently unknown if these electrophysiological alterations eventually revert or normalize at subsequent postnatal ages.

Frequency of mIPSCs

The lower mIPSC frequency found in *Lis1/sLis1* pyramidal neurons raises the possibility of the existence of alterations in GABAergic neurons since this parameter is dependent on the presynaptic cells. Recently, a decrease in the frequency of inhibitory synaptic currents in mice lacking *Dlx1* (Cobos et al., 2005) has been attributed to a lower number of cortical inhibitory neurons in the cortex of the mutant. Likewise, it is thus possible that the lower mIPSC frequency in *Lis1/sLis1* pyramidal neurons may be due to a reduced number of cortical GABAergic neurons in the cortex. Indeed, abnormal prenatal tangential neuronal migration has been reported in the *Lis1−/+* mouse (McManus et al., 2004). Alterations of transmitter release could also underlie the reduced mIPSC frequency which we observed; however, this possibility seems unlikely since release alterations are not present in other mutant of the *Lis1* gene (Fleck et al., 2000).

Both in WT and in *Lis1/sLis1* animals, mIPSC frequency increased from P7 to P30 postnatal ages. This increase in mIPSC frequency over the course of the first postnatal weeks has been observed in cells from several brain areas such as granule cells of

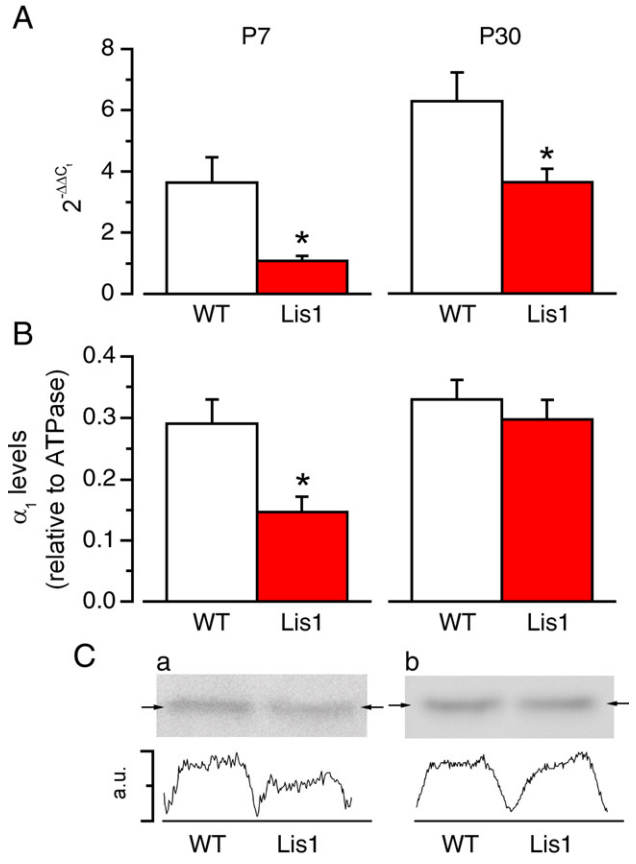

Fig. 5. Levels of α_1 subunit mRNA and protein at P7 and P30. (A) Levels of α_1 mRNA measured by qRT-PCR in WT brain tissue at P7 ($n=8$) and P30 ($n=6$), and in *Lis1/sLis1* at P7 ($n=8$) and P30 ($n=6$). The values of the $2^{(-\Delta\Delta Ct)}$ parameter were normalized to that measured in P7 *Lis1/sLis1*, where it was lowest in all cases. (B) α_1 protein levels in P7 (C) and P30 (D) brain tissue ($n=4$ in all cases). Levels of α_1 protein at each postnatal age were normalized with respect to those of the α_1 subunit of ATPase. $^*p<0.05$ in relation to WT; qRT-PCR data from P7 were compared using the Mann–Whitney Rank sum test. (C) Representative α_1 bands observed at P7 (a) and P30 (b); the plots below the bands show the profile of the density of each band measured between the arrows and expressed in arbitrary units (a.u.).

the dentate gyrus (Hollrigel and Soltesz, 1997), hippocampal pyramidal neurons (Banks et al., 2002), and cortical neurons (Dunning et al., 1999; Heinen et al., 2004; Luhmann and Prince, 1991) and has been associated with the massive increase in synaptogenesis which takes place during the first weeks of postnatal development (De Felipe et al., 1997). The degree of increase in mIPSC frequency from P7 to P30 was similar in *Lis1/sLis1* and in WT neurons (an increase of 2.14 times in *Lis1/sLis1* and 2.40 times in WT), suggesting that synapse formation takes place at a similar rate in the *Lis1/sLis1* and in the WT cortices.

Time course of the mIPSCs and the α_1 subunit of the GABA$_A$ receptor

The mIPSC decay phase time course was slower in *Lis1/sLis1* than in WT animals at P7 and P30, indicating that postsynaptic GABA$_A$ receptors of pyramidal neurons are altered in some way. This time course becomes faster during the first 2 weeks of postnatal development (Hollrigel and Soltesz, 1997) due to a change in the GABA$_A$ receptor subunit composition during early postnatal development of the cortex (Bosman et al., 2002; Dunning et al., 1999; Heinen et al., 2004). Most postsynaptic GABA$_A$ receptors are composed of two α, two β, and one γ subunit (Baumann et al., 2001; Farrar et al., 1999; Tretter et al., 1997). In most brain regions, postsynaptic GABA$_A$ receptors incorporate α_2 and/or α_3 subunits at birth (Fritschy et al., 1994; Laurie et al., 1992). These receptors mediate relatively long-lasting inhibitory postsynaptic currents (Bosman et al., 2002; Brussaard et al., 1997; Okada et al., 2000). However, during early postnatal development, the relative abundance of α_2 and α_3 diminishes (Fritschy et al., 1994; Heinen et al., 2004; Laurie et al., 1992). Simultaneously, the α_1 subunit, which is initially rare, is strongly upregulated, being the predominant α subunit in most brain regions during adulthood (Fritschy et al., 1994; Heinen et al., 2004; Laurie et al., 1992; Pirker et al., 2000). This process of subunit switching during the early postnatal development is altered in several circumstances, such as cortical malformations induced by freeze lesions in rat (Hablitz and DeFazio, 2000) or in rat models of temporal lobe epilepsy (Poulter et al., 1999). Synapses with mostly α_1-containing GABA$_A$ receptors mediate relatively short-lasting synaptic currents (Bosman et al., 2002; Goldstein et al., 2002; Koksma et al., 2003; Vicini et al., 2001). Although this developmental subunit switching and accompanied changes in GABAergic synaptic currents is a widespread phenomenon, its functional relevance is poorly understood.

We examined the presence of the α_1 subunit in GABA$_A$ receptors by testing the effect of zolpidem (a selective agonist of GABA$_A$ receptors containing α_1 subunits; Ruano et al., 1992; Renard et al., 1999; Vicini et al., 2001; Korpi et al., 2002) and by analyzing the levels of the corresponding α_1 mRNA and protein using qRT-PCR and Western blot respectively (Fig. 5). Our results show that zolpidem had a similar effect on mIPSCs at P7 and P30 in WT neurons, but it had no effect on mIPSC amplitude or decay time course at P7 in *Lis1/sLis1* neurons, increasing only the half amplitude decay time at P30. Together with our findings on the α_1 mRNA levels and α_1 protein levels (see Fig. 5), these results not only indicate lower levels of the α_1 subunit in the P7 *Lis1/sLis1* brain, but also that these lower levels of protein result in functional alterations, as revealed by the slower mIPSC and by the fact that zolpidem had no effect on mIPSCs at this postnatal age. In contrast, at P30 we found similar levels of α_1 protein in *Lis1/sLis1* and normal brains, but functional alterations persisted: the mIPSCs were slower and zolpidem had only a partial effect on mIPSCs (it increased the decay time but had no effect on the amplitude). The lower levels of α_1 protein at P7 could account for the differences in the mIPSC decay time course, but other mechanisms must be implicated at P30 since the α_1 protein levels were normal at this postnatal age. One possibility is that, in addition to the alterations in the levels of α_1 subunit that we have described, in the *Lis1/sLis1* mutant there are alterations in the levels or in the properties of the γ_2 subunit of the GABA$_A$ receptor. Zolpidem binds the benzodiazepine site of the GABA$_A$ receptor, which is formed at the junction of the α and γ subunits (Sigel, 2002; Ernst et al., 2003). Recently it has been shown that zolpidem does not increase the amplitude or the decay time of mIPSC recorded from Purkinje neurons (Cope et al., 2004) or from pyramidal neurons and interneurons of the hippocampus (Cope et al., 2005) in mice with a point mutation in the γ_2 subunit. Furthermore, in α_1 knockout mice, zolpidem still has some effect

(between 10% and 40% at concentrations of 30 or 300 nM) on the amplitude and decay time of hippocampal mIPSC (Goldstein et al., 2002). These data indicate that the effects of zolpidem on mIPSC are mediated also by the γ_2 subunit and open the possibility that other $GABA_A$ receptor subunits are implicated in the alterations observed in the mIPSCs from *Lis1/sLis1* mutant. Particularly, an alteration in the γ_2 subunit could explain the fact that at P30 the levels of α_1 were normal, but zolpidem had only effect on the decay time in *Lis1/sLis1* animals (see Fig. 4). It is important to note that, in the rat, the γ_2 subunit is not present in the early postnatal cortex (P10) while it reaches full levels of expression at P30 (Yu et al., 2006).

Interestingly, $GABA_A$ receptor functional alterations in the *Lis1/sLis1* cortex could be due to alterations in receptor subunit trafficking and/or incorporation into membrane receptors. For $GABA_A$ receptors, both mechanisms are dependent on the subunit binding to gephyrin which in turn interacts with dynein light chains (Fuhrmann et al., 2002). The LIS1 protein interacts with dynein and dynactin (Faulkner et al., 2000; Smith et al., 2000; Tai et al., 2002). Therefore, a mutation of the LIS1 protein could distort the processes of $GABA_A$ subunit trafficking and/or incorporation into functional membrane receptors. This abnormality would account not only for the functional alterations at P7 (slower time course and lack of effect of zolpidem), but also for the functional alterations found at P30, a postnatal age at which the α_1 protein levels were found to be normal. Alterations in $GABA_A$ receptor distribution between synaptic and extrasynaptic areas (receptor clustering in synapses) may also account for the observed phenomena since this distribution is also dependent on subunit binding to gephyrin. However, the fact that there were no differences in mIPSC amplitude between WT and *Lis1/sLis1* at P7 suggests that this possibility is less likely, at least at P7.

The intrinsic electrophysiological properties of pyramidal neurons were similar in WT and *Lis1/sLis1* animals, the only exception being the shorter duration of the action potential in *Lis1/sLis1* neurons at P30. Developmental changes in these properties from P7 to P30 were also similar in WT and *Lis1/sLis1* animals. These changes consisted of a decrease in input resistance, a shortening of the action potential, and an increase in the amplitude of the membrane potential and action potential, corroborating previous findings (Picken Bahrey and Moody, 2003; Zhang, 2004). Given that the activity of platelet-activating factor acetylhydrolase (PAF-AH, the enzyme whose β-subunit is the LIS1 protein; Hattori et al., 1994) is normal in the adult *Lis1/sLis1* brain (Cahana et al., 2001), the postnatal alterations in the *Lis1/sLis1* cortex which we report here are unlikely to be a consequence of elevated PAF levels. However, the possibility that PAF levels are still supra-normal during the initial postnatal days, thus contributing to the observed functional abnormalities, cannot be ruled out.

Experimental methods

We used *Lis1/sLis* mutant mice (on the MF-1 background) described in Cahana et al. (2001). Heterozygous mutants were used as the experimental subjects, and littermates were used as controls. For genotyping, DNA was extracted from a piece of mouse tail using a DNA isolation kit (Qiagen) and subjected to PCR as described in Cahana et al. (2001). Data are presented as the mean±s.e.m. Statistical comparisons were carried out using an unpaired, two-tailed Student's test or with the Mann–Whitney rank sum test when the samples did not pass the normality or equal variance tests; all statistics were performed with Sigma Stat 3.11 (Systat Software Inc.).

Electrophysiology

Cerebral slices

Electrophysiological experiments were performed on slices of neocortex prepared from 7- and 30-day-old wild type (WT) and *Lis1/sLis1* mice following methods described elsewhere (Geijo-Barrientos and Pastore, 1995; De la Peña and Geijo-Barrientos, 1996). Briefly, animals were killed by cervical dislocation, decapitated, and brains quickly excised and submerged in ice-cold extracellular solution (composition in mM: 124 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 10 glucose; pH 7.4 when saturated with 95% O_2+5% CO_2). A block of tissue, which included the frontal or the parietal cortex, was dissected out, and 4–6 300 μm thick coronal slices were cut using a vibratome (Leica VT-1000). Slices were maintained in a glass beaker where the tissue was submerged in extracellular solution continuously bubbled with 95% O_2+ 5% CO_2 at 37 °C for 30 min, and stored for at least 1 h at room temperature. One slice at a time was transferred to a submersion-type recording chamber and kept at 33–35 °C during recording time. The solutions used to bath the slices were fed into the recording chamber at a rate of 2–3 ml/min and were continuously bubbled with a gas mixture of 95% O_2+5% CO_2.

Cell identification and intracellular recording

Whole-cell recordings from neurons located in layers II/III of the frontal or parietal cortex were made under visual control in an upright microscope (Olympus BX50WI) equipped with Nomarski IR-DIC optics and a water immersion lens (40×). Pyramidal neurons were identified according to their morphological appearance in the living slice (Fig. 1C), and to their firing patterns in response to intracellular injection of depolarizing current pulses (McCormick et al., 1985; Connors and Gutnick, 1990; Kawaguchi, 1993; 1995); in some cases, the identification as pyramidal neurons was performed *a posteriori* by intracellular staining with biocytin (0.5% added to the intracellular solution). Patch pipettes were made from borosilicate glass (1.5 mm outer diameter, 0.86 mm inner diameter) and had a resistance of 3–5 MΩ when filled with the intracellular solution (composition in mM: 135 KCl, 10 EGTA, 10 HEPES, 2 $MgCl_2$, 4 Mg-ATP, 0.4 Na-GTP; pH 7.2 adjusted with KOH; 285–295 mosM). Recordings in current-clamp and/or voltage-clamp mode were obtained with a patch-clamp amplifier (Axopatch 200B, Axon Instruments). All neurons included in this study had resting membrane potentials more negative than −55 mV and fired tonically with overshooting action potentials in response to supra-threshold depolarizing current pulses. Miniature inhibitory spontaneous synaptic currents (mIPSCs) were recorded at a holding potential of −70 mV in the presence of 1 μM tetrodotoxin (TTX), 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM D(−)-2-amino-5-phosphonopentanoic acid (AP5). The mIPSCs recorded under these conditions were blocked by the application of 5 μM bicuculline, a selective blocker of $GABA_A$ receptors (tested in 6 neurons; data not shown). Due to the chloride-rich intracellular solution, the $GABA_A$ mediated responses were recorded as inward currents at −70 mV (Figs. 2A and B). No correction was made for the pipette junction potential. The series resistance ranged from 10 to 25 MΩ and was monitored throughout the recording. Voltage and current signals were filtered at 5 kHz and digitized at 10 kHz with a 16-bit resolution DA converter (Digidata 1322A, Axon Instruments, USA). Pulse generation and acquisition were controlled by the pClamp 8.0 software (Axon Instruments). mIPSCs were detected with the WinEDR v2.3.3 software (courtesy of Dr. J. Dempster, Strathclyde University, UK) by setting the detection threshold at −30 pA. The amplitude and the time course of the decay phase of the mIPSCs were measured from the averaged mIPSC detected in a 3-minute period of continuous stable recording. The decay phase of the averaged mIPSC was fitted to the sum of two exponential functions (using the Clampfit 8.0 fit tool), whereas the amplitude and the time constant of each component were combined to

obtain a single weighted time constant for each neuron; this weighted time constant was calculated as follows (where a_1 and a_2 the coefficients of both exponentials):

$$\tau_{\text{weighted}} = \tau_1(a_1/(a_1+a_2)) + \tau_2(a_2/(a_1+a_2))$$

All experiments were performed in a blind manner (animals were genotyped once experiments had been performed). All drugs used in the electrophysiological experiments were obtained from Sigma-Aldrich (USA), except TTX, CNQX and AP5, which were obtained from Tocris (Tocris Bioscience, UK). Zolpidem dissolved in extracellular solution was applied at 100 nM.

Quantitative RT-PCR and Western blot

For the quantitative RT-PCR (qRT-PCR) analysis, total RNA was isolated from mouse brain tissue (without cerebellum) using the TRIZOL (Total RNA Isolation Reagent, GIBCO BRL, USA) extraction procedure. This RNA was then assessed for purity and then quantified. Complementary DNA (cDNA) was obtained by reverse transcription (Superscript II, GIBCO BRL, Life Technologies, Gaithersburg, MD, USA) of total RNA (1 μg) using oligo-dT primers. Specific primers for the $GABA_A$ receptor α_1 subunit sequence were obtained commercially (Mm 00439040_m1, Applied Biosystems). DNA quantification was performed using a Perkin-Elmer AB PRISM 7700 sequence detection system (PE Biosystems, Foster City, CA) employing 40 cycles [50 °C 2 min; 95 °C 10 min; 40×(95 °C 15s; 60 °C 1 min)] with 1 μl cDNA per reaction (25 μl: Taqman® Universal PCR Mastermix; Applied Biosystems). Levels of gene expression in *Lis1/sLis1* and WT mice were calculated in relation to the eukaryotic 18S rRNA endogenous control (FAM/MGB Probe, Applied Biosystems) by: $E^{[Ct(\text{gene }\alpha1)-Ct(18S\ WT)]}$ where Ct (gene α_1, 18S) is the number of cycles at which the PCR products reached the predefined threshold value, and E is the amplification efficiency, which was taken as 2.

For Western blot experiments, brains (without cerebellum) from P7 and P30 mice were dissected and homogenized in 10 ml of homogenization buffer (50 mM Tris/HCl pH 7.5, 1 M NaCl, 5 mM EDTA, 5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride) at 4 °C in a Brinkman Polytron at 15,000 rpm. This procedure was repeated three times. Then, the homogenate was centrifuged at 100,000×*g* for 1 hr at 4 °C. The resulting pellet was recentrifuged in an equal volume of buffer, but with no NaCl on this occasion. The final pellet was resuspended in a small volume of buffer and solubilized with 1 ml of sample buffer for SDS–polyacrylamide gel electrophoresis (Laemmli, 1970). Samples were boiled for 5 min and subsequently used for Western blot assays. Proteins were measured by the method of Bradford (1976). For Western blot analysis, 10 μg of microsomal fraction proteins/lane was separated by 10% SDS–polyacrylamide gel electrophoresis and subsequently blotted as described by Towbin et al. (1979). After the transfer, nitrocellulose membranes were blocked for 1 h at room temperature with 5% dry milk in phosphate-buffered saline and incubated overnight either with an anti-$GABA_A$ receptor α_1 antibody (Abcam Ltd) (1:7000) or with an anti-ATPase α_1 subunit (1:1000) (Santa Cruz Biotechnology, Inc.). After incubating the secondary antibody at room temperature for 1 h, the bands were detected using ECL Plus Western blotting detection reagents (Amersham Biosciences). Finally, bands were visualized and quantified using a Bioimager (Fujifilm) and data analysis was performed using the Image Gauge 4.0 (Fujifilm) software.

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