

FAST CEREBELLAR OSCILLATION ASSOCIATED WITH ATAXIA IN A MOUSE MODEL OF ANGELMAN SYNDROME

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Abstract—Ataxia may result from various cerebellar cortex dysfunctions. It is included in the diagnostic criteria of Angelman syndrome, a human neurogenetic condition. In order to better understand the cerebellar dysfunction in this condition, we recorded *in vivo* cerebellar activity in a mouse model of Angelman syndrome produced by null mutation of the maternal *Ube3a* gene. We found fast oscillation (approximately 160 Hz) in the cerebellar cortex sustained by abnormally increased Purkinje cell firing rate and rhythmicity. This oscillation is inhibited by sensory stimulation and gap junction or GABA_A receptor blockers. A physiologically similar oscillation was previously found in mice lacking calcium-binding proteins that also present ataxia, but never in wild-type mice. We propose that fast oscillation in the cerebellar cortex is implicated in the cerebellar symptomatology of Angelman syndrome. © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: Angelman syndrome, *Ube3a*, cerebellum, oscillation, Purkinje cell.

Angelman syndrome (OMIM 105830) is characterized by mental retardation and motor dysfunction including ataxia. All patients with a molecular diagnosis of Angelman syndrome have a functional absence of the maternally inherited *UBE3A* gene, a normally imprinted gene located on chromosome 15q11–13 (Kishino et al., 1997). Cerebellar dysfunction, suggested since the original description of the syndrome (Angelman, 1965) has been confirmed by functional imaging (Holopainen et al., 2001) and movement studies (Dan et al., 2001; Dan and Cheron, 2004). Angelman syndrome mouse models with knockout maternal

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Abbreviations: CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; Cb^{-/-}, mice lacking calbindin; Cr^{-/-}, mice lacking calretinin; Cr^{-/-}Cb^{-/-}, mice lacking calretinin and calbindin; LFPO, local field potential oscillation; LFPOi, local field potential oscillation index; *Ube3a* m-/p+, mice with maternally inherited *Ube3a* deficiency; WT, wild type mice.

Ube3a (*Ube3a* m-/p+) have no morphologic cerebellar abnormalities (Jiang et al., 1998; Miura et al., 2002). However, they showed lack of *Ube3a* expression specifically in Purkinje cell layer (Miura et al., 2002) or cytoplasmic accumulation of *Ube3a* substrate in Purkinje cells (Jiang et al., 1998) and also showed ataxia (Jiang et al., 1998; Miura et al., 2002).

Purkinje cells are the sole output of the cerebellar cortex. Alteration of their *in vivo* firing has been reported in different model of ataxic animals (Sinclair et al., 1980; Schiffmann et al., 1999). Recently, we described an increased rhythmicity and synchrony of Purkinje cells associated with a fast oscillation of the local field potential in mice with inactivated calbindin (Cb^{-/-}) and/or calretinin (Cr^{-/-}) genes (Cheron et al., 2004a,b).

In order to better understand the cerebellar dysfunction in Angelman syndrome, we recorded *in vivo* cerebellar activity in *Ube3a* m-/p+ and wild type mice (WT).

EXPERIMENTAL PROCEDURES

Control and mutant mice

Mutants with the *Ube3a* null mutation were generated on a C57Bl/6 genetic background (Miura et al., 2002). Briefly, a cassette containing a picornaviral internal ribosome-entry site and a lacZneoR fusion gene was inserted at the site of deletion corresponding to *Ube3a* exons 15 and 16 which correspond to human *UBE3A*. This construct was linearized with *Sac*II then electroporated into ES cells line J1 (Li et al., 1992). Targeted clones were introduced into blastocyst of strain C57Bl/6J. The resulting chimeric mice showed high levels of germline transmission of the inactivated gene. Then, one founder male mouse was bred to a C57Bl/6J female. Mice used in the present experiments had been backcrossed for at least eight generations to C57Bl/6J. Genotyping of mice were carried out by Southern blotting and by polymerase chain reaction (PCR) of mouse tail DNA.

Single-unit recording in alert mice

Seventeen mice (eight *Ube3a* m-/p+, nine WT) mice aged 10–13 months, were prepared for chronic recording of neuronal activity in the cerebellum (Cheron et al., 2004a). Under general anesthesia with xylido-dihydrothiazin (Rompun; Bayer, Wuppertal, Germany; 7 mg/kg) and ketamine (Ketalar; Pfizer, Groton; 100 mg/kg), two small bolts were cemented to the skull to immobilize the head during the experimental session. The surface of the uvula of the cerebellum was exposed by reflecting the muscles overlying the cisterna magna and a small hole was drilled in the skull. The dura was removed over lobules 9a and 9b and over Crus IIA for whisker stimulation experiments and an acrylic recording chamber constructed around the hole. The cerebellum was explored with glass micropipette (1.5–5.0 MΩ impedance). After amplification (1000–2000×) and bandpass filtering (10 Hz–10 kHz), the recordings were stored on 4 mm digital audio tapes and transferred to a Pentium III personal computer with

analog-to-digital converter boards (Power 1401, CED). The recorded data were digitized continuously at 10 kHz and treated off-line by Spike 2 CED software. Animals were kept in accordance with the guidelines established by the ethical committee of UMH (Mons, Belgium) for the care and use of laboratory animals. Efforts were made to minimize the number of animals used and their suffering. Criteria for Purkinje cells recording and data analysis were the same as those used in a previous study (Cheron et al., 2004a). Autocorrelation histograms with a time bin of 1 ms were plotted for simple spike firing from single Purkinje cell. We quantified the strength of the oscillation with a rhythm index (Sugihara et al., 1995). Briefly, peaks and valleys were recognized if their heights and depths exceeded the mean baseline level \pm S.D. (measured at time lags of 250–300 ms). The rhythm index was then defined by the following formula:

$$\text{rhythm index} = a_1/z + b_1/z + a_2/z + b_2/z + \dots$$

in which a_i ($i=1,2,\dots$) is the absolute value of the difference between the height of the i th peak and baseline level, b_i ($i=1,2,\dots$) is the absolute value of the difference between the height of the i th valley and baseline level, and z was the difference between the height of the zero-time bin and the baseline level.

Multiunit recording in alert mice

Multiunit recordings along the frontal plane were performed by means of seven linearly arranged, quartz-insulated, platinum–tungsten fiber–microelectrodes with 250 μm inter-electrode spacing (Eckhorn and Thomas, 1993).

Local field potential analysis. Local field potential oscillations (LFPO) were analyzed by the wave-triggered average technique (Steriade et al., 1998) and fast Fourier transform. A LFPO index (LFPOi) was computed by dividing the maximum amplitude of the power spectrum peak by the total area of the power spectrum.

In vivo microinjection. Injection micropipettes, drawn from calibrated 0.275 μm internal diameter glass tubing (tip OD: 250 μm) were filled with either a solution of 27 mM SR95531 (gabazine, GABA_A antagonist) or 48 mM carbenoxolone (gap junction blocker). Saline solution was injected in control experiments. Injections were carried out using an air pressure system (air pulses of 10 ms of duration; $n=20$).

Tactile stimulation of the whisker region. Facial dermatomes of the whisker regions were stimulated by calibrated air puffs delivered by an air pressure system (Picospritzer II) with an air pressure at the source of 2.6 bar, 40 psi. Air puffs (20 ms of duration) were applied through a glass pipette (tip diameter of 2 mm). The tip was located 1 cm away from the skin of the whisker region at a lateral angle of 50° with respect to the midline of the head.

Results are expressed as mean \pm S.D. Cross-correlation analysis was performed using the time series module of Statistica 6.0, Statsoft. Means are compared by ANOVA test performed on Statistica 6.0, Statsoft.

RESULTS

Emergence of fast oscillation in *Ube3a* m-/-p+

Spontaneous spindle-shaped (5.6 ± 1.5 episodes/s) LFPO (maximal amplitude 0.45 ± 0.22 mV, mean frequency 158.9 ± 30.1 Hz) was found throughout the explored regions (vermis, uvula, nodulus) in *Ube3a* m-/-p+ mice (Fig. 1). In contrast, LFPO was not recorded in WT mice. Therefore, we compared LFPOi of *Ube3a* m-/-p+ with those measured in *Cb*^{-/-}*Cr*^{-/-} mice in which fast cerebellar oscillation was first reported (Cheron et al., 2004a). Oscil-

lation indices (12.5 ± 7.8 in *Ube3a* m-/-p+ versus 13.2 ± 6.2 in *Cr*^{-/-}*Cb*^{-/-} (Cheron et al., 2004a) and topography were similar to those observed in *Cr*^{-/-}*Cb*^{-/-} mice, including spatial coherence along the same parallel fiber beam (Fig. 1a,b). Spindles appeared simultaneously at the different loci. Fig. 1C illustrates the cross-correlation analysis between signals 1 and 2, 2 and 3, and 3 and 4 showing for each LFPO pair significant correlation (mean coefficient of 0.78 ± 0.11) peaking at a lag of 0 ms.

Altered Purkinje cell firing in *Ube3a* m-/-p+

Purkinje cell simple spike spontaneous firing rate was higher in mutants than in WT animals (Fig. 2a,c), whereas there was no difference in spontaneous complex spike firing rate (Table 1). Durations of complex spikes and of the subsequent pause in simple spike firing were significantly reduced. In WT mice, Purkinje cells typically fired tonically at an irregular rate (approximately 50 Hz) while in mutant mice, increased firing appeared highly rhythmic in 50% of Purkinje cells (Fig. 2b,d).

One-sided peak counts of simple spike autocorrelation were higher in *Ube3a* m-/-p+ (3.0 ± 2.4 , $n=30$) than in WT mice (0.8 ± 0.1 , $n=36$; $P < 0.0001$). Rhythm index was higher in mutant mice (0.17 ± 0.14) than in WT (0.03 ± 0.02 ; $P < 0.0001$).

Inhibition of fast oscillation by pharmacological agents

Given the role of gap junction and GABA_A transmission in fast brain rhythms, we studied the effect of carbenoxolone and gabazine (SR95531) microinjections (Fig. 3). Both agents reversibly reduced LFPO amplitude. Five minutes after carbenoxolone injection (Fig. 3a), LFPOi was reduced to $25.5 \pm 18.9\%$ of pre-injection values, and 5 min after gabazine injection (Fig. 3b), it was reduced to $40.0 \pm 15.6\%$. The time course of LFPOi was similar in both agents, with recovery of baseline values within 30 min after injection (Fig. 3c). In contrast, saline injection produced no significant LFPOi alteration.

Inhibition of fast oscillation by whisker stimulation

Given the involvement of Purkinje cells in sensorimotor processing (Bower and Woolston, 1983), we stimulated the whisker region and examined the effect on the LFPO in order to approach its response to afferent input. Fig. 4 illustrates the suppression of the spontaneous LFPO recorded at three loci situated along the parallel fiber beam in response to an air puff directed to the whisker. In this illustration, LFPO is shown filtered with a low-pass digital filter (500 Hz) and averaged ($n=10$) with the trigger adjusted to the first wave occurring after the air puff. LFPO suppression was consistently recorded along the parallel fiber beam. The mean duration of LFPO suppression was 195 ± 63 ms.

DISCUSSION

We report here the emergence of fast (160 Hz) oscillation in the cerebellum of a mouse model of Angelman syn-

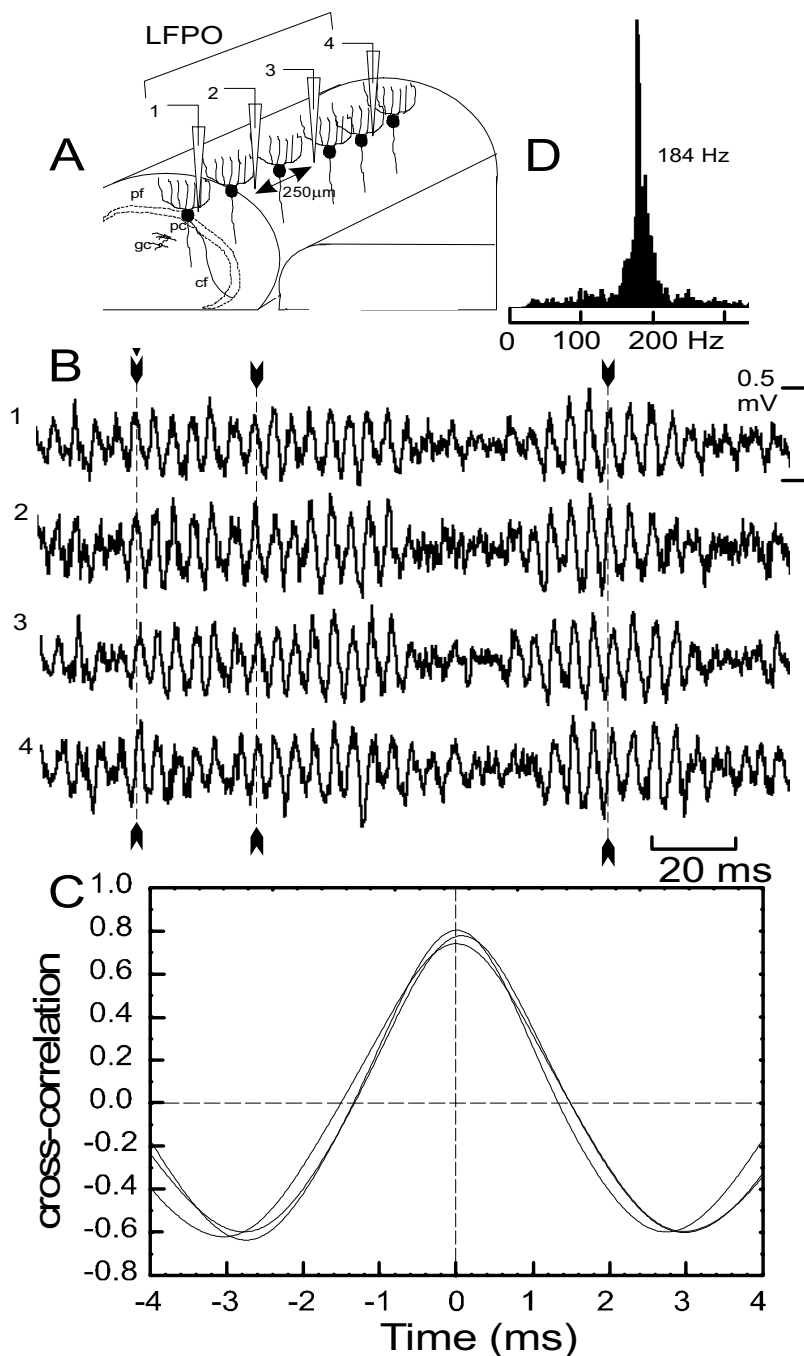


Fig. 1. Emergence of high-frequency LFPO in the cerebellum of *Ube3a* *m-p/+* mice. (a) Sketch of microelectrode placement along parallel fiber beam. Adjacent electrodes are distant by 250 μm . (b) Sample LFPO records at four sites. Dashed lines indicate synchronization. (c) Cross-correlation analysis between signals 1 and 2, 2 and 3, and 3 and 4. (d) Fast-Fourier transform of recording labeled 1 in (b) peaked at 184 Hz.

drome. This LFPO is physiologically similar to that described in *Cr^{-/-}*, *Cb^{-/-}* and in *Cr^{-/-}Cb^{-/-}* mice (Cheron et al., 2004a). In these three groups of knockout mice, LFPO is related to increased simple spike firing and rhythmicity, decreased complex spike duration and shortened subsequent pause in simple spike firing.

The inhibition of fast LFPO by sensory stimulation may be related to the induced modification of simple spike firing (Bower and Woolston, 1983; Cheron et al., 2004b). This

behavior shows similarities with cerebral “resting” rhythmic activities of wakefulness arresting to sensory or motor information, such as cortical α and μ rhythms (Donoghue et al., 1998).

LFPO suppression by carbenoxolone and gabazine points to the contribution of inhibitory molecular interneurons densely connected through dendrodendritic gap junctions (Sotelo and Llinás, 1972; Mann-Metzer and Yarom, 1999) and fast GABA_A receptor synapses (Kondo and Marty, 1998;

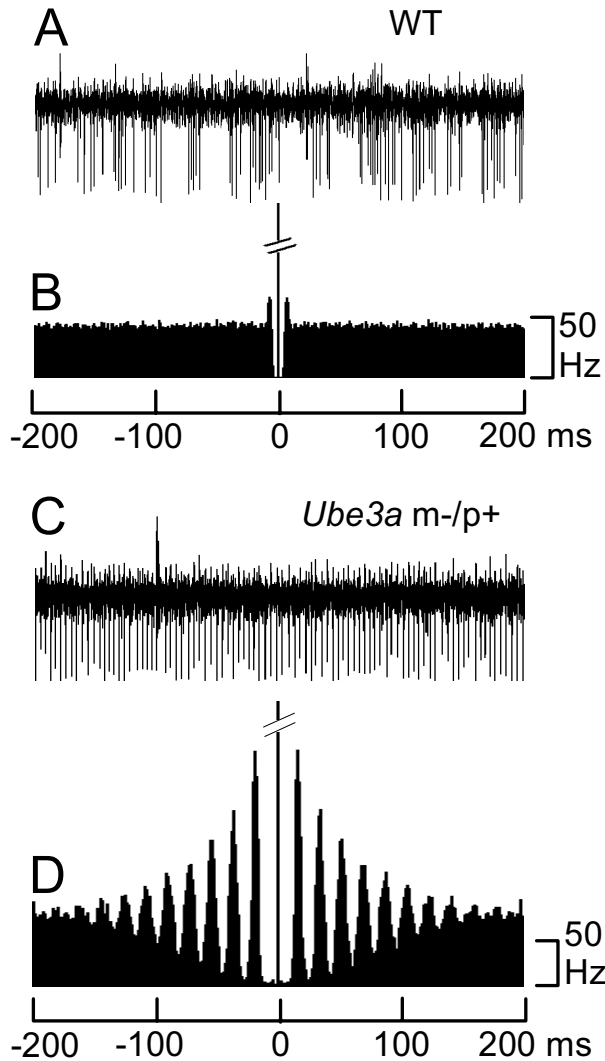


Fig. 2. Firing behavior of WT Purkinje cells (PC)(a) and *Ube3a* *m*⁻/*p*⁺ (c). Irregular firing (mean 50 Hz) in WT (a). Simple spike autocorrelogram in WT (b) and *Ube3a* *m*⁻/*p*⁺ (d).

Carter and Regehr, 2002;). In contrast, in the hippocampus interneurons did not seem to participate in the 200 Hz oscillations (also blocked by both carbenoxolone and GABA_A receptor antagonist; Draguhn et al., 1998). A more complex mechanism could be involved in the generation of this high

Table 1. Purkinje cell firing behavior in alert WT and *Ube3a* *m*⁻/*p*⁺ mice^a

Parameter	WT, <i>n</i> =86 cells	<i>Ube3a</i> <i>m</i> ⁻ / <i>p</i> ⁺ , <i>n</i> =61 cells
SSf, Hz	51.8±21.6	87.3±27.8*
CSf, Hz	0.49±0.26	0.54±0.30
CSd, ms	10.3±1.5	8.1±2.3*
Pause, ms	20.4±8.0	11.9±7.2*

^a CSd, complex spike duration; CSf complex spike firing rate; SSf, simple spike firing rate.

* *P*<0.00001 as compared to age-matched, WT animals; one-way ANOVA.

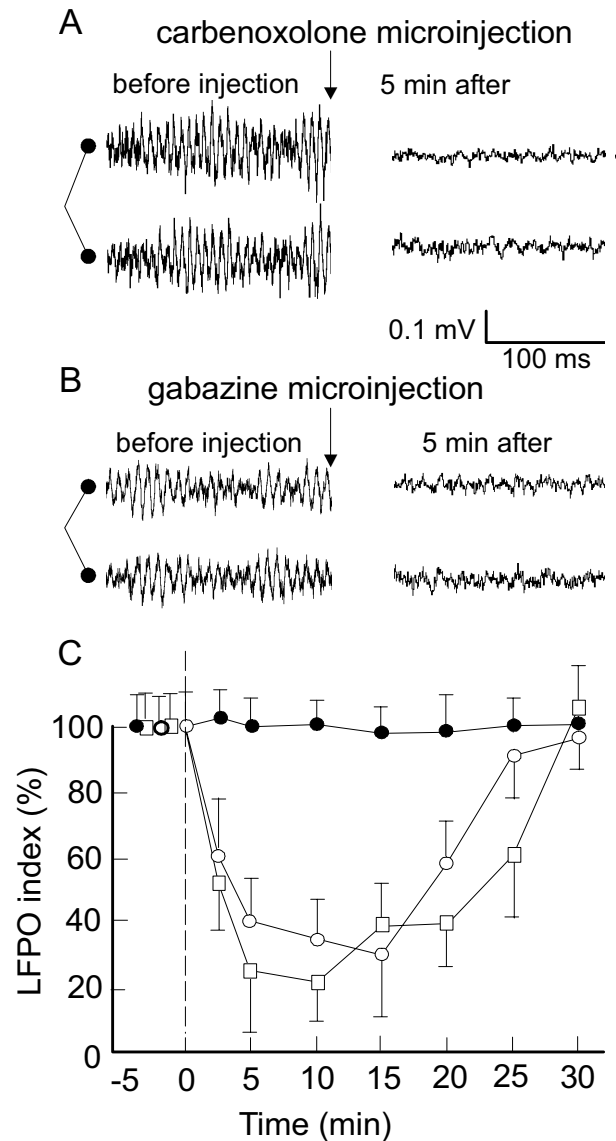


Fig. 3. Effects of carbenoxolone and gabazine microinjections on the 160 Hz LFPO in *Ube3a* *m*⁻/*p*⁺ mice. Raw LFPO recordings with 250 μ m distanced microelectrodes before (left) and 5 min after (right) carbenoxolone (a) or gabazine (b) microinjections. Time course of LFPOi (c) with respect to microinjection of carbenoxolone (open squares), gabazine (open circles) and saline (filled circles).

frequency oscillation which is, to our knowledge, the only physiological precedent that is similar to the cerebellar oscillation. Experimental and theoretical studies demonstrated that the collective behavior of gap-junction coupling between axons of pyramidal cells results in high-frequency field oscillation (>100 Hz; Traub et al., 2003a,b). This fast oscillation occurs as a consequence of random activity within the axonal plexus and is uncovered when all chemical synapses are blocked. It was proposed that ectopic spike generation at a low rate (0.05–1/s) is able to sustain such high-frequency oscillation (Traub et al., 2003a,b). The interplay between this latter and γ oscillations seems to be governed by a dual role of the GABA_A receptor. Nonsomatic GABA_A receptor activation enhances this collective oscillation, whereas perisomatic

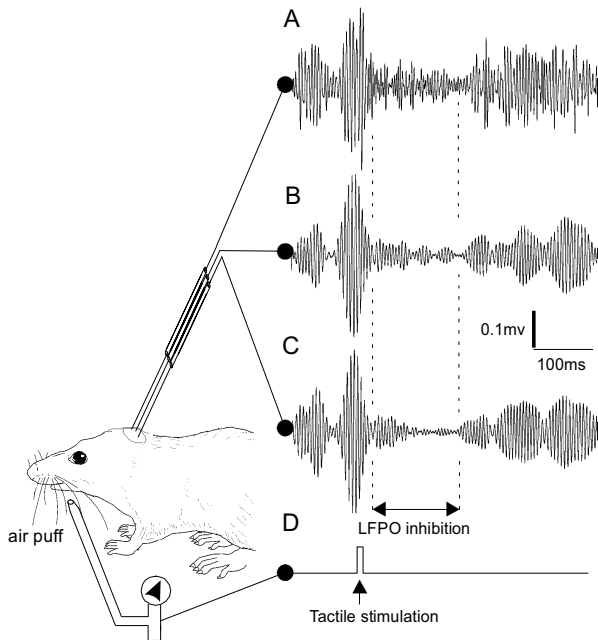


Fig. 4. LFPO inhibition during cutaneous stimulation of the whisker region. (a–c) Low-pass filtered (200 Hz) averaged LFPO recorded in three distant loci (inter-electrode distance of 250 μm). The trigger for averaging was adjusted to the first wave occurring after the air puff (d).

GABA_A receptors, that are activated by interneuron input, phase this random activity at γ rhythm (Traub et al., 2003a,b). At present, it is not clear whether similar mechanisms can be involved in the oscillation in the cerebellum. The presence of a number of gap-junction channel-forming proteins has been suggested in the Purkinje cell layer (Simburger et al., 1997; Teubner et al., 2000, 2001), although some of these findings have been disputed (Meier et al., 2002; Odermatt et al., 2003). Recently, a new class of gap-junction proteins, namely pannexins, has been found to be expressed in Purkinje cell layer (Bruzzone et al., 2003), but their possible role in axo-axonal coupling is yet to be established. In addition, ultrastructural demonstration of axo-axonal coupling is complicated by the fact that a very low incidence of coupling may suffice to generate fast oscillation (Traub et al., 1999).

The molecular mechanisms by which *Ube3a* inactivation results in Angelman syndrome and in cerebellar LFPO are still unclear. However, different mechanisms may relate *Ube3a* deficiency and GABA_A transmission. The product of *Ube3a* acts along the ubiquitin-associated proteasome pathway (Kleijnen et al., 2000), where it might affect the regulation of GABA_A receptors containing $\beta 3$ subunit (Dan et al., 2004).

The neurophysiological LFPO similarities between mutant mice lacking *Ube3a* and calcium binding proteins might suggest that these calcium binding proteins are deficient in *Ube3a* m-/p+ mice. However, immunocytochemical staining demonstrated normal calbindin expression in the Purkinje cell of *Ube3a* m-/p+ mice (Jiang et al., 1998). Another mechanism might implicate Ca²⁺ homeostasis and GABA_A transmission. Intrinsic neuronal Ca²⁺ conductances and intracellular [Ca²⁺] homeostasis

are considered as crucial partners in the emergence of neuronal network activity, including oscillations (Llinás, 1988). In *Cb*^{-/-} mice, specific alterations in the Purkinje cell dendritic compartment have been revealed by the detection of increased synaptically evoked Ca²⁺ transients (Airaksinen et al., 1997; Barski et al., 2003). In *Ube3a* m-/p+ mice, deficits in hippocampal long-term potentiation have recently been related to diminished activity of the Ca²⁺/calmodulin-dependent protein kinase II (CaMKII; Weeber et al., 2003). In the Purkinje cell, CaMKII is critically involved in the signaling cascade regulating the long-term potentiation of GABA_A receptors (Kawaguchi and Hirano, 2002). The increased spontaneous Purkinje cell firing found in *Ube3a* m-/p+ mice may result from impairment in this regulation process. Given the specific reduction in CaMKII activity demonstrated in *Ube3a* m-/p+ mouse hippocampus (Weeber et al., 2003), similar derangement may be reasonably suspected in Purkinje cells, leading to alteration of the rebound potentiation of the GABA_A receptors (Kano et al., 1992, 1996). In *Cb*^{-/-} mice, the increase in Ca²⁺ transient influx in cells (Airaksinen et al., 1997; Barski et al., 2003) could form a link between change in Ca²⁺ homeostasis and activation of a gap junction-coupled-network producing 160 Hz oscillations, as gating of gap junctions can be modified by increased intracellular [Ca²⁺] (De Pina-Benabou et al., 2001).

The role of the 160 Hz LFPO is currently unclear. It could lead to ataxia or reflect a compensatory mechanism allowing the cerebellum to function despite abnormal Purkinje cell firing (Cheron et al., 2004b). In this view, LFPO synchrony could act as a spatiotemporal filter sharpening the action of selected rostrocaudal modules of the cerebellum (Voogd and Glickstein, 1998). The focal mossy fiber input related to the cutaneous stimulation probably overcomes the generalized state of synchronous activation of Purkinje cell populations. LFPO arrest is produced by transient excitation–inhibition or inhibition–excitation response of the Purkinje cell (Cheron et al., 2004a).

To date, fast LFPO has been reported in four different knockout mice (*Cb*^{-/-}, *Cr*^{-/-}, *Cb*^{-/-}*Cr*^{-/-} and *Ube3a* m-/p+). All these mice show developmental ataxia (Airaksinen et al., 1997; Schiffmann et al., 1999; Miura et al., 2002). Such high-frequency oscillation sustained by increased simple spike firing and rhythmicity has never been described in WT or in non-ataxic mice. This may have major implications for future therapeutic targeting, as fast LFPO may be inhibited by different pharmacological options.

In conclusion, 40 years after the first description of Angelman syndrome, the present report provides the first neurophysiological evidence of cerebellar dysfunction in an animal model of Angelman syndrome. This is consistent with a network mechanism implicating gap junctions and GABA_A transmission. This may be highly relevant to the understanding and therapeutic targeting in cerebellar symptoms of human Angelman syndrome.

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