Neuroscience 130 (2005) 631–637

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

G. CHERON,[a,b]* L. SERVAIS,[a,c] J. WAGSTAFF[d] AND B. DAN[b,c]

[a]*Laboratory of Electrophysiology, Université Mons-Hainaut, 7000 Mons, Belgium*

[b]*Laboratory of Movement Neurophysiology, CP 168, 24 Avenue P. Héger, Université Libre de Bruxelles, Brussels, Belgium*

[c]*Department of Neurology, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium*

[d]*Department of Pediatrics and Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA, USA*

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.

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 *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; Pzifer, 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

*Correspondence to: G. Cheron, Laboratory of Movement Biomechanics, Université Libre de Bruxelles, CP 168, 24 Avenue P. Héger, Université Libre de Bruxelles, 1000 Brussels, Belgium. Tel: +32-2650-2187; fax: +32-650-3566.
E-mail address: gcheron@ulb.ac.be (G. Cheron).

Abbreviations: CaMKII, Ca^{2+}/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.

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2004.09.013

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±S.D. (measured at time lags of 250–300 ms). The rhythm index was then defined by the following formula:

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

in which a_i (i=1,2,. . .) is the absolute value of the difference between the height of the ith peak and baseline level, b_i (i=1,2,. . .) is the absolute value of the difference between the height of the ith 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 trough 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±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 LFPO*i* of *Ube3a* m−/p+ with those measured in $Cb^{-/-}Cr^{-/-}$ mice in which fast cerebellar oscillation was first reported (Cheron et al., 2004a). Oscillation 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 autocorrelogram 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±18.9% of pre-injection values, and 5 min after gabazine injection (Fig. 3b), it was reduced to 40.0±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-