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PURKINJE CELL RHYTHMICITY AND SYNCHRONICITY DURING MODULATION OF FAST CEREBELLAR OSCILLATION

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Abstract—Fast (~160 Hz) cerebellar oscillation has been recently described in different models of ataxic mice, such as mice lacking calcium-binding proteins and in a mouse model of Angelman syndrome. Among them, calretinin–calbindin double knockout mice constitute the best model for evaluating fast oscillations *in vivo*. The cerebellum of these mice may present long-lasting episodes of very strong and stable local field potential oscillation alternating with the normal non-oscillating state. Spontaneous firing of the Purkinje cells in wild type and double knockout mice largely differs. Indeed, the Purkinje cell firing of the oscillating mutant is characterized by an increased rate and rhythmicity and by the emergence of synchronicity along the parallel fiber axis. To better understand the driving role played by these different parameters on fast cerebellar oscillation, we simultaneously recorded Purkinje cells and local field potential during the induction of general anesthesia by ketamine or pentobarbitone. Both drugs significantly increased Purkinje cell rhythmicity in the absence of oscillation, but they did not lead to Purkinje cell synchronization or to the emergence of fast oscillation. During fast oscillation episodes, ketamine abolished Purkinje cell synchronicity and inhibited fast oscillation. In contrast, pentobarbitone facilitated fast oscillation, induced and increased Purkinje cell synchronicity.

We propose that fast cerebellar oscillation is due to the synchronous rhythmic firing of Purkinje cell populations and is facilitated by positive feedback whereby the oscillating field further phase-locks recruited Purkinje cells onto the same rhythmic firing pattern. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: cerebellum, oscillation, rhythmicity, synchronicity, ketamine, pentobarbitone.

The understanding of interactions between neuronal network oscillation and single neuronal firing is central to the study of oscillation genesis and function. Local field potential oscillations (LFPO) have been described in many regions of the mammalian brain, such as the thalamus (Steriade et al., 1996), cerebellum (Pellerin and Lamarre, 1997), hippocampus (Whittington et al., 1995; Faulkner et al., 1999), and other parts of the cerebral cortex (Steriade et al., 1993; Collins et al., 2001; Grenier et al., 2003). They may support physiological processes (Hartmann and Bower, 1998; Baker et al., 1999), result from pharmacological impregnation (Fisahn et al., 1998) or reflect pathological conditions, such as seizures (Khazipov and Holmes, 2003; Grenier et al., 2003) or ataxia (Cheron et al., 2004, 2005).

In the cerebellar cortex, fast LFPO (130–260 Hz) was first described in mice lacking calretinin and/or calbindin (Cheron et al., 2004), two proteins involved in calcium buffering in granule and Purkinje cells, respectively. A similar fast cerebellar LFPO is also present in a mouse model of Angelman syndrome (Cheron et al., 2005), and in mice lacking parvalbumin (Servais et al., 2005). All these mice with fast cerebellar LFPO (calbindin, calretinin, calbindin/calretinin and Ube3a deficient mice) are ataxic (Airaksinen et al., 1997; Schiffmann et al., 1999; Miura et al., 2002). In contrast, wild type (WT) mice do not present cerebellar LFPO suggesting that this oscillation is a cause or a marker of cerebellar dysfunction.

The cerebellum of mice with LFPO may switch between long-lasting periods of fast oscillation and of the normal non-oscillating state. Although the factors leading to the spontaneous emergence of such fast oscillatory episodes are not known, simple and complex spikes of Purkinje cell populations have been demonstrated to be tightly phase-locked to LFPO. In mice with fast oscillation, the simple spikes of Purkinje cells recorded close to the oscillating field present increased rhythmicity, firing rate and synchronicity (Cheron et al., 2004). Different hypotheses attempt to explain the emergence of LFPO and Purkinje cell synchronicity in mice lacking calcium-binding proteins. First, increased excitability of granule (Gall et al., 2003) and/or Purkinje cells could lead to increased Purkinje cell firing rate and to increased rhythmicity and synchronicity of these cells, possibly giving rise to fast oscillation. Indeed, Stratton et al. (1988) have reported a relationship between Purkinje cell rhythmicity and firing rate. Fast LFPO would thus be considered as a side effect of increased simple spike firing rate, which would be the primary cause of ataxia and other cerebellar electrophysiological abnormalities. Another view is that fast oscillation itself would be the primary cause of Purkinje cell synchronization in a high rhythmic frequency range. This fast oscillation would then "trap" Purkinje cells, rendering their firing less adaptable, possibly leading to ataxia. These two

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Abbreviations: APV, D,L-2-amino-5-phosphonovaleric acid; $Cb^{-/-}Cr^{-/-}$, mice lacking calbindin and calretinin; LFPO, local field potential oscillation; NMDA, N-methyl-D-aspartate; WT, wild type mice.

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mechanisms may coexist. In neocortical fast LFPO, Grenier et al. (2003) proposed a vicious feedback loop in which very fast oscillations in field potentials reflect the synchronous action of neocortical neurons and help to generate and synchronize action potentials in adjacent neurons through electrical interactions (Jefferys, 1995).

In order to test these hypotheses, simultaneous recordings of fast oscillation and Purkinje cells are required during the emergence, inhibition or facilitation of LFPO. Cerebellar microinjections (Cheron et al., 2004, 2005; Servais et al., 2005) have demonstrated the involvement of $GABA_A$ and N-methyl-D-aspartate (NMDA) receptors in maintaining cerebellar LFPO. However, long-lasting stable multiple recording of single-unit Purkinje cells in alert mice during local microinjections is extremely difficult to perform. Moreover, the drug concentration at the different recording sites is currently impossible to standardize during microinjection. Therefore, we studied fast LFPO and Purkinje cell firing before and during general anesthesia induced by pentobarbitone (a $GABA_A$ receptor positive modulator) or ketamine (a NMDA receptor antagonist). Given their modes of action, these substances were expected to respectively facilitate and inhibit fast LFPO. Both drugs are known to diffuse rapidly and homogenously in mouse brain (Saubermann et al., 1974; Blednov and Simpson, 1999). The aim of this study was to characterize Purkinje cell firing rate, rhythmicity and synchronicity during the inhibition or the facilitation of LFPO.

EXPERIMENTAL PROCEDURES

Mice

Sex- and age- (3–8 months) matched WT and calretinin/calbindin double knockout mice ($Cb^{-/-}Cr^{-/-}$) (Cheron et al., 2004) generated on a mixed 129×C57Bl/6 genetic background were used in all experiments.

Surgical preparation for recording

Mice were anesthetized with xylido-dihydrothiazin (Rompun®, Bayer, Germany, 10 mg/kg) and ketamine (Ketalar®, CEVA, Belgium, 100 mg/kg). Animals were administered an additional dose of xylido-dihydrothiazin (3 mg/kg) and ketamine (30 mg/kg) if they presented agitation or markedly increased respiration or heart rate during the procedure. In addition, local anesthesia with 0.5 ml lidocaine 20 mg/ml+Adrenaline 1:80,000 (Xylocaine®, Astra Zeneca, UK) was administered s.c. during soft tissue removal. Two small bolts were cemented to the skull to immobilize the head during the recording sessions and a silver reference electrode was placed on the surface of the parietal cortex. An acrylic recording chamber was constructed around a posterior craniostomy, covered by a thin layer of bone wax (Ethicon®, Johnson and Johnson, USA) before the recording sessions. An i.p. catheter was placed and fixed to the skin. Twenty-four hours after anesthesia, alert mice were immobilized for the recording session. The dura mater was removed locally above the vermis. Recordings were performed in lobules IV–VIII, and the location of the electrodes (depth and lobule) was noted. We did not select a specific lobule for the different experiments, but during multiple recordings, electrodes were placed in the same lobule.

Single-unit recordings

Single-unit recordings were performed with glass micropipettes filled with NaCl 0.2 M (1.5–5 MΩ of impedance). A neural signal was considered as originating from a Purkinje cell if it presented two types of spiking activities: simple spikes characterized by single depolarization (300–800 μs) occurring between 20 and 200 Hz and complex spikes characterized by an initial fast depolarization (300–600 μs) followed by smaller and relatively constant wavelets. It was considered that simple and complex spikes originated from the same Purkinje cell when a transient pause (~20 ms) in simple spike firing followed each complex spike.

Multi-unit recordings

Multiple recordings along the same parallel fiber axis were performed by means of seven linearly arranged, quartz-insulated, platinum-tungsten fiber-microelectrodes (outer and shaft diameter of 80 μm and 25 μm, respectively) with 250-μm inter-electrode spacing. Each microelectrode was mounted into a stretched elastic rubber tube enabling proper positioning via DC-micromotors (resolution of 0.27 μm) (Eckhorn and Thomas, 1993).

Drug injection

After the recording of oscillation and/or Purkinje cell activity lasting a minimum of 120 s, ketamine 100 mg/kg, dilution 1/5 with NaCl 0.9% and pentobarbitone (Nembutal®, CEVA) 30 mg/kg, dilution 1/20 with NaCl 0.9% were slowly (~30 s) injected through the i.p. catheter. No mice underwent more than one injection on the same day and no more than a total of two injections. Recording and analysis were performed until 15 min after the injection unless otherwise mentioned. At the end of the recording session, mice were killed by a lethal dose of pentobarbitone through the i.p. catheter.

Microinjections

Pentobarbitone (24 mM), ketamine (42 mM) or D,L-2-amino-5-phosphonovaleric acid (APV) (Sigma, France) (a NMDA-specific antagonist) (42 mM) was injected through a micropipette, drawn from calibrated 1.16 mm internal diameter glass tubing (internal diameter: 30 μm), using an air pressure system (Picospritzer II), pulse of 5 ms, five pulses. Each pulse delivered a volume of 0.125 μL. No mice underwent more than one microinjection the same day and more than a total of three microinjections.

Data analysis

All recordings were performed with a bandwidth of 0.01 kHz to 10 kHz. They were stored on 4 mm digital audio tapes (Sony PCM-R500) 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. Off-line analysis and illustrations were performed using the Spike 2 CED software. The discrimination between Purkinje cell simple and complex spikes was performed by the same software (waveform recognition) and controlled visually before analysis.

The rhythmic frequency was defined as the reciprocal of the latency of the first peak in the autocorrelogram of simple spike firing (width=1 s, bin size=0.2 ms). Consequently, rhythmic frequency could not be determined on flat autocorrelograms. The strength of the rhythmicity was quantified with a rhythm index measured on the simple spike autocorrelogram (120 s-lasting recording, width=1 s, bin size=1 ms) (Sugihara et al., 1995; Lang et al., 1999). Briefly, the height and depth of all peaks and valleys that were significantly different from the baseline and occurred at specific latencies with regard to the initial peak were summed. The sum was divided by the total number of spikes in the recording. In the autocorrelograms with no significant peaks and valleys, a