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Spectral analysis of cerebellar activity after acute brain injury in anesthetized rats

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Abstract. We investigated cerebellar electrocortical activity before and after unilateral brain injury in anesthetized rats. Spectral analysis of cerebellar activity was obtained by Fast Fourier Transformation. There was a dominance of delta frequency range, while the wide gamma range presented no more than 5% of the total mean power spectra of cerebellar activity before brain injury. A few minutes after brain injury and within the first 90 minutes, there was a decrease of total mean power spectra and a relative decrease of delta range power to about 30%, some increase of beta range, and an increase of gamma range to 20–25%. Relative increase of gamma range in the cerebellar mean power spectra was still present 120 min after the brain injury, while other changes started to diminish. We suggest that spectral changes within slow and fast (gamma) frequency ranges of cerebellar activity may be indicators of the brain state after acute injury.

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INTRODUCTION

The effects of brain injury on cerebellum have received relatively low attention. Traumatic brain injury represents a combination of immediate mechanical disruption of brain tissue and secondary delayed mechanisms that develop over a period of hours to days and months following the injury. Cells from various brain regions respond differently to mechanical injury (as recently shown by Slemmer et al. 2003) and Purkinje neurons are most affected by cerebellar trauma. Besides known connections of the cerebro-cerebellar system (Allen and Tsukuhara 1974), changes in the cerebellar electrocortical activity, as indicators of different pathological states, are not elucidated. Moreover, the importance of fast (gamma) activity not only at cerebral but also at cerebellar cortical levels is not completely understood in the context of sensory motor control (Bullock and Achimowicz 1994). Presence of theta rhythm in cerebellar activity is connected to information processing in the thalamocorticocerebellar system of unrestrained rats (Hartmann and Bower 1998) and could indicate states of expectancy and attention (Karhu and Tesche 1998). The aim of our study was to investigate the effect of acute brain injury on spectral changes of cerebellar cortical activity. A preliminary account of this study appeared in abstract form (Culic et al. 2002).

METHODS

Experimental animals and surgical procedure

The experiments were performed on 10 male Wistar rats at the age of 2.5–3 months. All procedures were done in accordance with the guidelines of the National Institutes of Health (Bethesda) and the Institute for Biological Research (Belgrade) for the care and use of laboratory animals. The surgery was done under pentobarbitalsodium (Serva, Heidelberg; initial dose 35 mg/kg, intraperitoneally) anesthesia. Animals were mounted in a stereotaxic apparatus. Partial round shaped craniotomies were made over the parietal cerebral cortex (P: 2–2.5; L/R: 2–2.5) and paravermal cerebellar cortex (P: 11.5–12.0; L/R: 1.5–2.0), according to stereotaxic coordinates (in millimeters) with respect to bregma, for positioning the recording electrodes. The strength of pedal withdrawal reflex, body immobility grade and respiratory rhythm were used to assess the depth of anesthesia (deep during surgery and light during recording procedure). Subsequent doses of anesthetic (~8 mg/kg) were added if necessary. A discrete area of the left parietal cortex (P: 2–2.5; L: 2–2.5; H: 0–0.5) was injured through dura and pia mater by a sterile needle (21G). The volume of the cone-like injury was about 0.08 mm^3. Bleeding was stopped within several seconds and the rate of respiratory movements soon normalized spontaneously.

After completing the recording procedure and suturing muscles and skin over the wound, each of the eight animals was kept with great care, including prevention of infection and free access to food and water during a 12-hour light/dark cycle. With no visible motor disabilities after a few days, the animals were used for the next 6–9 months in other semi-chronic experiments

Recording procedure and data analysis

Local field potentials of the paravermal cerebellar cortex and parietal cerebral cortex were bipolarly recorded by epidurally positioned silver ball (diameter: 0.5 mm) electrodes through marked craniotomies, with a reference of silver wire (diameter: 0.2 mm), laid over frontal bone and temporal muscles. Cerebellar electrocortical activity was amplified and filtered (Neurolog, Digitimer, England; low pass DC, 1 kHz, 50 Hz notch). Analog to digital conversion of the recorded signals was performed at the sampling rate of 256 Hz and stored for off-line analysis. Each recording sequence lasted 240 s and pauses between acquisition sessions lasted 5–10 min. Spectral analysis of the recorded signals, during sequences of 80 s to 240 s (divided into epochs of 8 s), was obtained by Fast Fourier Transformation. Mean power spectra (of 10 to 30 epochs) of cortical activity were obtained in frequency ranges of 4 Hz up to 128 Hz.

A one-way repeated-measures ANOVA and Tukey *post-hoc* test were used to determine significance before and after brain injury of relative cerebellar mean power spectra changes in the slow and fast frequency ranges. Brain injury effects on the relative mean power spectra of cerebellar activity at a particular time (up to 120 min) after injury were also tested by Mann-Whitney test. Spectral changes of parietal cerebral cortical activity were the focus of another study (Culic et al. 2003).

Histological verifications

Two experimental animals were sacrificed under deep anesthesia after the first recording procedure, in

order to obtain histological verification of acute injury. Thereafter, the brains of experimental animals were extracted, fixed and prepared for histological analysis. Control of native histological preparations showed only discrete lesions of designate size at the left parietal cortex. The study on the extension of necrosis and gliosis of directly injured and indirectly affected brain regions, at both cerebral and cerebellar levels, is in progress.

RESULTS

The control state of anesthetized rats, before brain injury, was characterized by a predominance of slow, delta range frequency (0.1–4.0 Hz) in the mean total power spectra at the cerebellar level. The initial stable state of each experimental animal was confirmed by obtaining at least two very similar mean total power spectra within 30 minutes before injury. Absolute mean power of cerebellar activity was several-fold lower than we found at the cerebral level (Culic et al. 2003). More specifically, control cerebellar mean power within delta range (0.1–4.0 Hz) was ~50%, theta (4.1–12 Hz) ~35%, beta (12.1–32 Hz) ~10% and gamma (32.1–128.0 Hz) ~5%. A typical example of cerebellar electrocortical activity before injury, including its absolute and relative mean total power spectra, is shown in Fig 1.

Desynchronization in cerebellar electrocortical activity occurred a few minutes after the brain injury. Thus, a few minutes and up to 30 min after the brain injury, there was a decrease in the total mean power of cerebellar activity. Relative decrease could be noticed in delta (0.1–4.0 Hz), slight increase in beta (12.1–32.0 Hz) and mild increase in gamma (32.1–128.0 Hz) frequency ranges, as shown in Fig. 2.

It should be mentioned that there was a large inter-experiment variability of relative changes of certain cerebellar power spectra bands before as well as after the injury. Analysis of variance (one way with repeated measures) showed, after pooling data of 10 animals in experimental conditions: before, 0–30 min, 30–60 min, 60–90 min and 90–120 min after acute trauma, $F(4, 36)$, that brain injury was a factor inducing significant changes in the relative mean power spectra of delta range (F=5.2195, P<0.01), beta (F=2.8415, P<0.05), and particularly gamma range (F=16.8532, P<0.0001), but not in theta (F=1.4563, P=0.2358). Multiple comparisons by Tukey test of data at different times after acute injury showed also that a period of 120 min was a

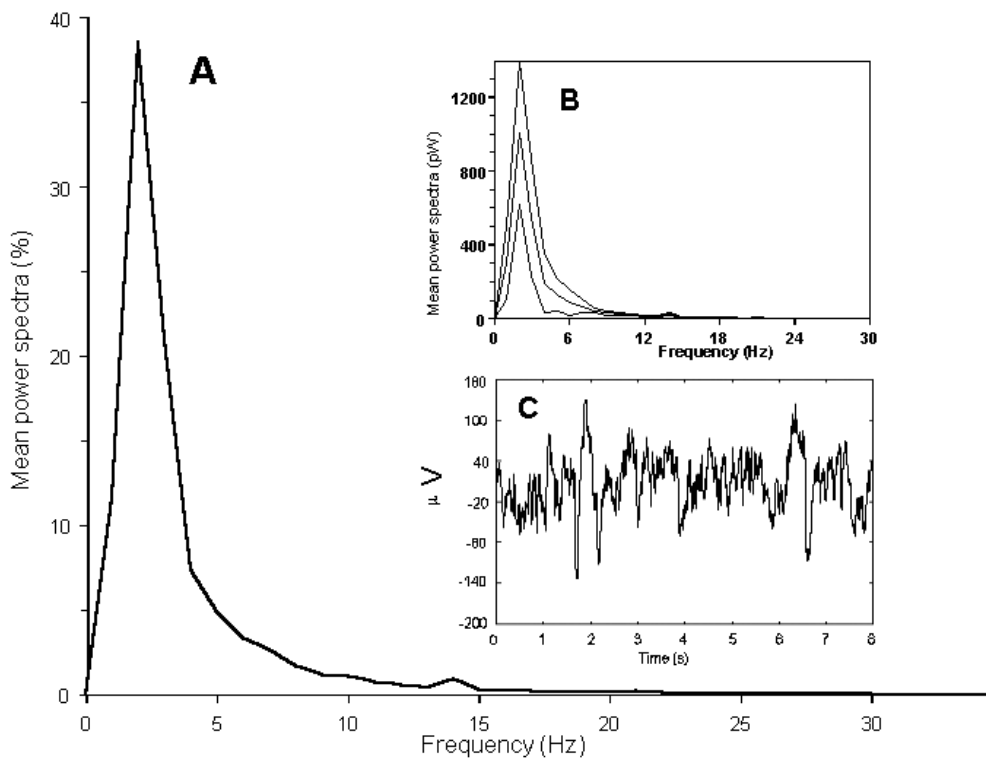

Fig. 1. Cerebellar electrocorticogram 10 min before brain injury: an epoch of 8 s (C), its corresponding relative spectrum (A), and the total mean power spectrum ± SD (B) of ten epochs

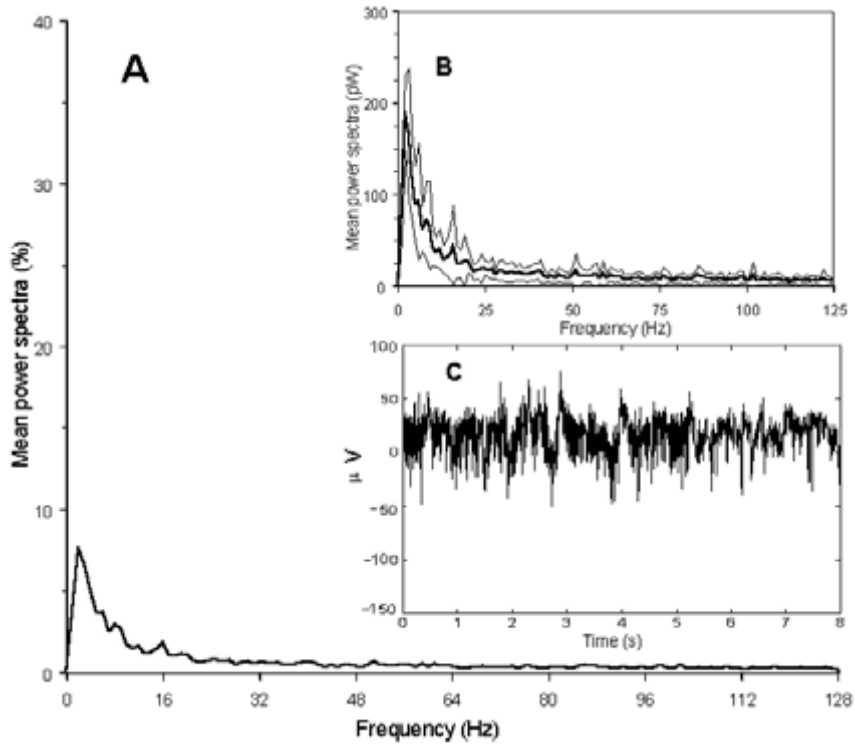

Fig. 2. Cerebellar electrocorticogram 20 min after brain injury (the same experimental animal as in Fig.1): an epoch of 8 s (C), its corresponding relative spectrum (A) and the total mean power spectrum ± SD (B) of ten epochs

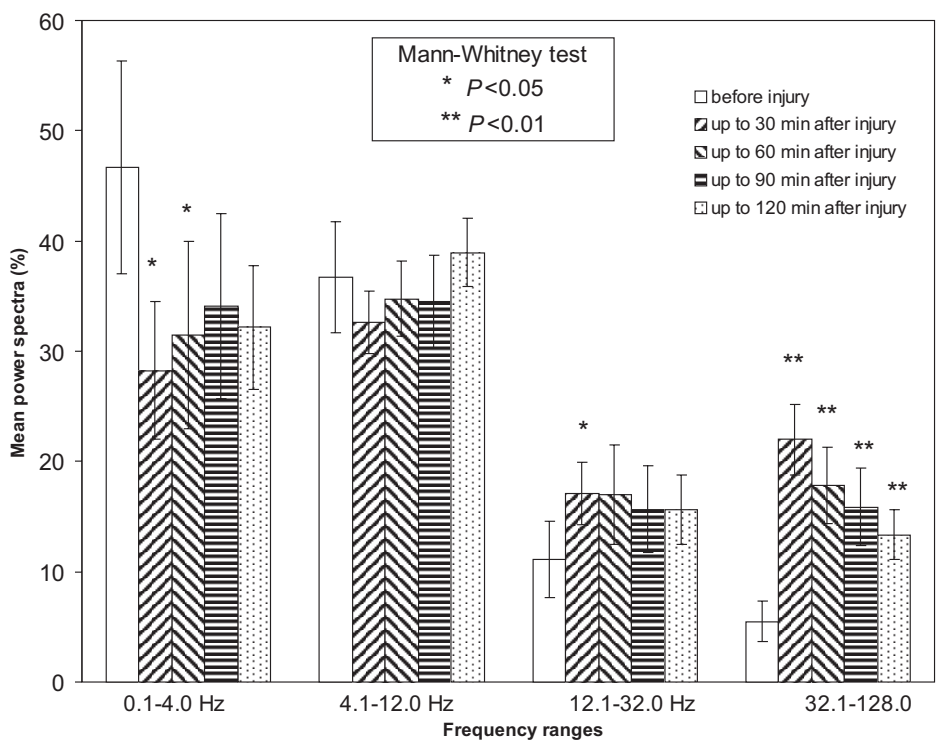

Fig. 3. Relative mean power spectra of cerebellar activity in delta, theta, beta and gamma frequency ranges of 10 rats, before and during 30, 60, 90 and 120 min after injury differed when compared to the control values before injury, at noted probabilities, using Mann-Whitney test. The averages (± SD indicated by bar) of the cerebellar relative mean power spectra after brain injury are noted.

factor keeping significant changes only in gamma range (at $P<0.007$).

Some spectral changes of cerebellar electrocortical activity after acute brain injury were present at certain times, but at various levels of significance, compared with the control (before injury) values. The effects of acute brain injury on cerebellar electrocortical activity started to diminish within the first hours after injury. Beta range of cerebellar electrocortical activity started to restore within the first hour, and delta range during the second hour after the lesion (Fig. 3). Delta range power gradually increased up to about 35–40% within the second hour after brain injury, but gamma range of the cerebellar mean power spectra remained at ~15% (increased in respect to the control), even 120 min after the acute injury.

DISCUSSION

Our results show that there was a decrease of the total mean power, relative decrease of slow and increase of fast (gamma) activity in cerebellar paravermal cortex, after discrete acute brain injury. We may consider several aspects of cerebellar response to acute brain injury that connect rhythmic brain activity to functional states: similarity of cerebellar and other brain region reactions to similar events, significance of activity as a sign of injury and the possible underlying cellular mechanisms.

Cerebellar activity was characterized by a similar decrease in the mean total power spectra after discrete acute brain injury as the parietal cortical one (Culic et al. 2003) but the relative increase of gamma range was greater at the cerebellar than the cerebral level. The remote effects of flattened cerebral electrocortical activity in acute brain injury are not known but cortical spreading depression was noted in animal models of focal cerebral ischemia and it was suggested that it could be associated with protective (Kobayashi et al. 1995) or harmful effects (Busch et al. 1996). A decrease of power in bilateral cortical and hippocampal EEG was revealed after unilateral lesion of the ventral tegmental area, which led to a decrease in regional dopaminergic transmission (Jurkowlaniec et al. 2003). The effects of acute and chronic brain injury are different. The involvement of cerebellum and the noradrenergic system was only suggested in some animal studies on the recovery of locomotor function after extensive unilateral sensorimotor cortical injury (Boyeson et al. 1993, Hovda and Feeney 1984).

Our preliminary results (Kalauzi et al. 2004) indicate that the coefficient of linear correlation between changes of relative gamma power spectra and fractal dimensions of left cerebral, right cerebral, left cerebellar and right cerebellar signals was relatively high. There is still controversy (Choi et al. 1999, Juhasz et al. 1997) about relative slow and fast oscillations in the great body of evidence on spectral changes in EEG with massive ischemic cerebral lesions, but there is no mention of cerebellar reactions. It should be pointed out that the type, site, chronic state and extension of brain injury varied in different studies, while we focused on an acute, discrete brain injury in the current study.

We did not find significant changes of cerebellar activity in theta ranges, as our animals were anesthetized and the recorded sites were not restricted to the granule cell layer. A substantial level of disagreement exists about the effects of anesthesia at various neural levels and assessment of depth of anesthesia is still not adequately standardized (Antunes et al. 2003, Dougherty et al. 1997). It was obvious that the suggested contribution of theta oscillations to sensory information processing, as described for rat cerebellar granular layer (Hartmann and Bower 1998), rat olfactory cerebral cortex (Bower 1996) and somatosensory cortex of awake monkeys (Murthy and Fetz 1996), could not be revealed in our anesthetized preparation.

We can only speculate on the relevance of gamma oscillations, as studied by other authors in various brain structures. Gamma oscillations (within a wide range of 30–150 Hz) of parahippocampal activity (Hirai et al. 1999) were present in electroencephalograms of epileptic patients. The profile of post-injury power spectrum within the gamma frequency range, which we obtained at the cerebellar level, does not indicate the appearance of one or several oscillations, but points toward a disordered, noise-like activity. This could be a spectral reflection of cortical trauma.

The pacemaker mechanisms in individual neurons, responsible for cerebellar synchronization, remained unclear. It is possible that interneurons, coupled with GABAergic connections and paired with enhanced excitability, are responsible for gamma activity. There is an increase in the simple spike firing rate of Purkinje cells (Servais et al. 2003) in the cerebellum of alert mice lacking calbindin. It is supposed that cerebellar theta oscillations are more usually associated with inferior olivary neurons and their climbing fiber projections (Sasaki et al. 1989) than with granule cells. The study of

oscillation in the firing rate of Purkinje cells (Culic et al. 2001) suggested that intensified noradrenergic input induced an increase of slow power spectra, while intensified excitatory input caused an increase in the fast (gamma) range. However, the highly rhythmic nature of Purkinje cell simple spike discharge was remarkably stable across states of sleep and wakefulness (Nitz and Tononi 2002). A short duration of high frequency (150–200 Hz) local field potentials could be reproduced in a feed-forward model of cerebellar cortex (Maex et al. 2002). There is also evidence (Chen and Nitz 2002) for slow (2–10 Hz) and gamma (30–50 Hz) frequency coherence between spike trains and local field potentials in the cerebellum. In order to reveal the mechanisms of spectral changes in cerebellar activity it would be important to determine whether discharge of Purkinje and other neurons is phase related to local field potential oscillations in the model of acute brain injury, as well as in the chronic model.

CONCLUSION

After a discrete cerebral injury in anesthetized rats, mean power spectra of cerebellar cortical activity exhibited a decrease in total, relative decrease in delta and relative increase in gamma frequency ranges. These spectral changes have shown that the cerebellum is very susceptible to discrete brain injury and changes in its activity could be an indicator of the acute state.

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REFERENCES

Allen G, Tsukuhara N (1974) Cerebrocerebellar communication system. Physiol Rev 54: 957–1006.

Antunes LM, Roughan JV, Flecknell PA (2003) Effects of different propofol infusion rates on EEG activity and AEP responses in rats. J Vet Pharmacol Therap 26: 369–376.

Bower JM (1996) Reverse Engineering the Nervous System: An in Vivo, in Vitro and in Compute Approach to Understanding the Mammalian Olfactory System. An Introduction to Neural and Electronic Networks. Academic, San Diego, p. 3–29.

Boyeson MG, Krobert KA, Scherer PJ, Grade CM (1993) Reinstatement of motor deficit in recovered brain-injured animals: the role of cerebellar norepinephrine. Restor Neurol Neurosci 5: 293–290.

Bullock TH, Achimowicz JZ (1994) A comparative survey of oscillatory brain activity, especially gamma-band rhythms. In: Oscillatory Event Related Brain Dynamics (Pantev C, Elbert T, Lukenhoner B, eds.). Plenum Pub Corp., New York, p.11–26.

Busch E, Gyngell ML, Eis M, Hoehn Berlage M, Hossmann KA (1996) Potassium-induced cortical spreading depression during focal cerebral ischemia in rats: contribution to lesion growth assessed by diffusion-weighted NMR and biochemical imaging. J Cereb Blood Flow Metab 16: 1090–1099.

Chen Y, Nitz DA (2002) Evidence for slow (2–10 Hz) and gamma frequency coherence between spike trains and local field potentials in the cerebellum. Neurocomputing 52–54: 159–164.

Choi KG, Cho EK, Chae SH, Kim ES, Kim JS (1999) Spectral and bispectral EEG analysis in acute unilateral ischemic stroke patients. Neurol Psychiatry Brain Res 7: 9–14.

Culic M, Saponjic J, Jankovic B, Kalauzi A, Jovanovic A (2001) Slow oscillations of the Purkinje cell firing rate induced by electrical stimulation of the locus coeruleus in rats. Neurophysiology 33: 55–59.

Culic M, Martac L, Grbic G, Spasic S, Kalauzi A, Jankovic B (2002) Occurrence of gamma (33–128 Hz) activity in the cerebellum of anesthetized rats. FENS Abstr 1: A 192.1.

Culic M, Grbic G, Martac Blanusa Lj, Spasic S, Jankovic B, Rankovic R (2003). Slow and fast oscillations in the activity of parietal cortex after brain injury. In: From Basic Motor Control to Functional Recovery III (Gantchev N, ed.). St. Kliment Ohridski University Press, Sofia, p. 41–45.

Dougherty PM, Li YL, Lenz FA, Rowland L, Mittman S (1997) Correlation of effects of general anesthetics on somatosensory neurons in the primate thalamus and cortical EEG power. J Neurophysiol 70: 1375–1392.

Hartmann MJ, Bower JA (1998) Oscillatory activity in the cerebellar hemispheres of unrestrained rats. J Neurophysiol 80: 1598–1604.

Hirai N, Uchida S, Maehara T, Okubo Y, Shimizu H (1999) Enhanced gamma (30–150 Hz) frequency in the human medial temporal lobe. Neuroscience 90: 1149–1155.

Hovda DA, Feeney DM (1984) Amphetamine with experience promotes recovery of locomotor function after unilateral frontal cortex injury in cat. Brain Res 298: 358–361.

Juhasz C, Kamondi A, Szirmai I (1997) Spectra EEG analysis following hemispheric stroke. Acta Neurol Scand 96: 397–400.

Jurkowlaniec E, Tokarski J, Trojniar W (2003) Effects of unilateral ibotenate lesions of the ventral tegmental area on cortical and hippocampal EEG in freely behaving rats. Acta Neurobiol Exp (Wars) 63: 369–375.

Kalauzi A, Spasic S, Culic M, Grbic G, Martac Lj (2004) Correlation between fractal dimension and power spectra after unilateral cerebral injury in rat. FENS Abtrs 2: A.199.9.

Karhu J, Tesche C (1998) Characterization of hippocampal and cerebellar activity. http//boojum.hut.fi/research/brain/ar98hippo.html

Kobayashi S, Harris VA, Welsh FA (1995) Spreading depression induces tolerance of cortical neurons to ischemia in rat brain. J Cereb Blood Flow Metab 15: 721–727.

Maex R, Solinas S, DeSchutter E (2002) A feed-forward network model of high-frequency oscillations in local field potentials. FENS Abstr. 1: A 126.11

Murthy VN, Fetz EE (1996) Synchronization of neurons during local field potential oscillations in sensorimotor cortex of awake monkeys. J Neurophysiol 76: 3968–3982.

Nitz D, Tononi G (2002) Tonic rhythmic activity of rat cerebellar neurons. Exp Brain Res 146: 265–270.

Sasaki K, Bower JM Llinas R (1989) Multiple Purkinje cell recording in rodent cerebellar cortex. Eur J Neurosci 1: 572–586.

Servais L, De Saedeleer C, Schwaller B, Schiffmann SN, Cheron G (2003) Emergence of a double high-frequency oscillation in the cerebellum of mice lacking parvalbumin and calbindin. Symposium: Creating Coordination in the Cerebellum, Catania, Abstracts, p.62.

Slemmer EJ, De Zeeuw CI, Weber JT (2003) Cell death, gliosis and elevated S-100B protein in cerebellar cultures following mechanically induced trauma. Symposium: Creating Coordination in the Cerebellum, Catania, Abstracts, p.65.

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