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Interspike Background Activity in Extracelullarly Recorded Purkinje Neurons: Spectral Analysis

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Summary

The aim of this study was to investigate the spectral characteristics of Purkinje cell interspike background activity caused by the occurrence of particular action potentials or by electrically induced enhancement of cerebellar inhibitory and excitatory input drive. Spontaneously active Purkinje neurons were extracellularly recorded in anesthetized rats before and after cessation of stimulation from the inferior olive (IO) or locus coeruleus (LC). After A/D conversion (30 kHz), direct spectral analysis of extracted interspike background activity was done. Our results have shown that, in contrast to simple spikes, the occurrence of complex spikes induces changes in the spectra of interspike background activity. The different spectral changes of interspike background activity induced by LC and IO stimulation also indicated the importance of this extracellularly recorded phenomenon.

Key words

Spectral analysis • Purkinje neurons • Interspike background activity

Introduction

The discharge pattern of cerebellar Purkinje neurons is unique in the central nervous system due to two different action potentials, consisting of either simple or complex spikes (Eccles *et al.* 1967). The complex spikes occur at a low frequency, as a consequence of direct excitatory input *via* climbing fibers from the inferior olive (IO), whereas simple spikes occur at much higher frequencies and are driven by excitatory inputs from a number of brainstem nuclei, e.g. *via* mossy fibers-granule cells-parallel fibers (Eccles *et al.* 1967). It is still not clear how complex spikes modify simple spike activity (Sato *et al*. 1992, Miall *et al*. 1998) and how the excitation of Purkinje cells can be normalized (De Schutter 1995). The input to Purkinje neurons arrives from the locus coeruleus (LC) through noradrenergic fibers (Olson and Fuxe 1971). There is evidence in favor of dominant noradrenergic control over spontaneous activity of Purkinje neurons and the inhibition induced by noradrenaline is stronger than that of any other biogenic amine (Moises *et al*. 1979). Electrostimulation of LC induces a pause in Purkinje neuronal discharges (Bickford-Wimer *et al*. 1991) and their inhibition in the rat model of epilepsy (Ćulić *et al*. 1996). All the above mentioned studies analyzed the firing rates of Purkinje cell spikes, but there is no evidence about the nature of Purkinje neuronal interspike background activity intermingled between the spikes.

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The aim of our study was to investigate the spectral changes of interspike background activity caused by typical spike occurrence and especially by the electrically induced augmentation of the inhibitory input from LC or excitatory input from IO. Our preliminary studies indicated the interspike background activity of Purkinje cells as an important part of extracellularly recorded unit activity (Šaponjić *et al.* 1998, 1999).

Material and Methods

The acute experiments were performed under Nembutal (Sigma, USA) anesthesia (initial dose of 35-40 mg/kg, i.p.) on adult Wistar male rats. The experimental animals were subjected to a 12 h light-dark cycle and were housed with free access to food and water. The rats were prepared for extracellular recordings of cerebellar Purkinje neuronal activity with glass microelectrodes (resistance 6-10 MΩ). Special precautions were undertaken in order to avoid pain and suffering: all wounds were carefully infiltrated with 2 % lidocaine. The heart rate and EEG of the parietal cortex were continuously monitored. The surgical procedure was described in detail elsewhere (Ćulić *et al.* 1994). There were two experimental groups of animals. In one group of rats, a twisted stainless steel electrode was positioned according to the stereotaxic coordinates (Paxinos and Watson 1982) in IO (P: 12.5; L: 0.8; H: 10.8). In the other group of rats the stimulation electrode was positioned in LC (P: 9.5; L: 1.2; H: 7.5). The stimulation electrodes in both groups were positioned contralateral to the recording site. The parameters of LC stimulation were 0.2 ms pulse duration, frequency 10 Hz, amplitude 15 V and 15 s trains, whereas those for IO stimulation were 0.2 ms pulse duration, frequency 10 Hz, 3 V amplitude and 15 s trains. Electrophysiologically identified spontaneously active Purkinje neurons of the posterior paravermal cortex (P: 11.5 mm; R: 3 mm, up to 6 mm from the cerebellar surface) were recorded on line for 30-120 s. The usual signal-to-noise ratio in our experimental conditions was greater than 3. Unit activity was observed before and 1, 6, 11 and 15 min after the cessation of electrical stimulation in each experimental group, under light anesthesia (maintained by subsequent 5 mg/kg, i.p. doses of Nembutal if necessary, every 45-60 min). Analog to digital conversion of the recording signal was performed at a sampling rate of 30 000 Hz. After extraction of interspike background activity, without recognizable spikes from the digital signal, direct spectral analysis by Fast Fourier Transformation (FFT) was done within the frequency range up to 1500 Hz. Samples of 0.016 s of interspike background activity were used immediately before and after the appearance of a particular spike, while longer samples (0.200 s) were used when we tested the enhanced inhibitory or excitatory influences. Statistical analysis of time spectral amplitude modules of minimums, maximums and sums was carried out within the total (up to 1500 Hz) and in selected frequency ranges: very low (VL = 0-249 Hz), low (L = 250-499 Hz), high (H = 500-749 Hz), very high (VH = 750-1500 Hz). Student's paired t-test and single factor ANOVA were used for evaluation of particular spike influences on spectra of single neuron interspike background activity. Five time series of interspike background activity (overall 1 s), extracted from the whole recorded signal (30 s), were used as representative for each 8 neurons, before and after electrical stimulation. Thus, the 40 spectral module sums from 8 neurons in all frequency ranges before and after electrical stimulation were analyzed by the paired t-test.

Results

Spectral analysis of interspike background activity of 20 Purkinje neurons revealed that the occurrence of simple spikes did not cause changes in the spectral characteristics of interspike background activity. An example of background activity spectra before and after the appearance of a simple spike is shown in Figures 1A and 1B**.** There was a significant decrease of average module sums only in the high frequency range of background activity spectra after simple spikes ($t=1.85$; $p=0.04$), but it was not sufficient to produce changes in the whole range. On the contrary, there were changes in the spectra of background activity before and after complex spikes (Figs 1C and 1D). This included a significant decrease of average amplitude module sums in the total range ($t=4.00$; $p=0.0003$) due to decreases in the low ($t= 5.42$; $p<0.0001$), high ($t=3.11$; $p=0.003$) and very high ($t=2.86$; $p=0.005$) frequency ranges. There was also an increase in the very low range ($t=2,36$; $p=0.01$). Besides the decrease of module in the whole range, the occurrence of complex spikes induced slower frequency components in the spectra of interspike background activity. When we analyzed the spectral changes of background activity independent of a particular type of spike occurrence, but only with respect to the spike as a unique event, we obtained a significant increase of average module sums only in the total frequency range of spectra before the complex spike ($F=3.06$, $p=0.03$, $Fcrit=2.69$).

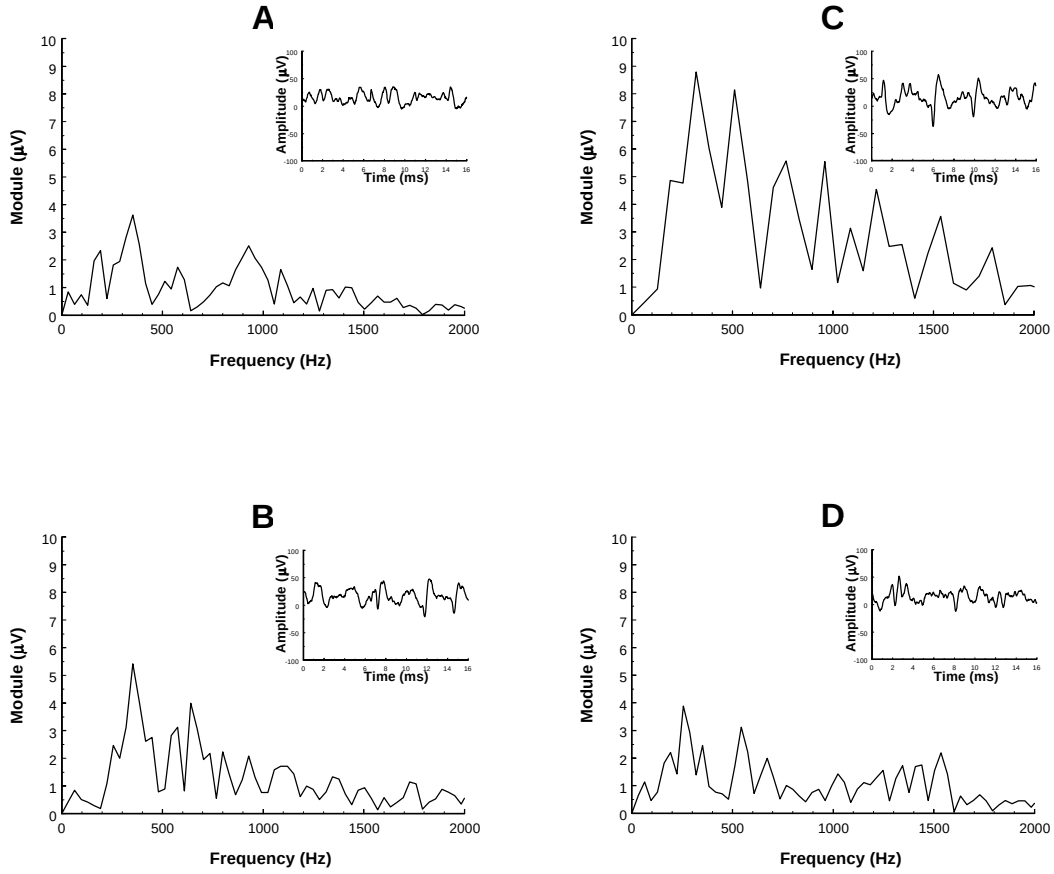

Fig. 1. *Changes of interspike background activity spectra caused by spike occurrence in one Purkinje cell: spectra of interspike background activity immediately before a simple (A) and complex spike (C) with their corresponding analog signals of 0.016 s; spectra of interspike background activity immediately after a simple (B) and complex (D) spike with their corresponding analog signals of 0.016 s.*

Besides the reversible increase in frequency of complex spikes, electrical stimulation of IO induced changes in the spectra of interspike background activity. We compared the spectra of background activity in 8 Purkinje neurons 1, 6, 11 and 16 min after the cessation of IO stimulation with the spectra before stimulation. Our results showed that there was a significant increase of the spectral average module sums in the whole frequency range only 6 min after IO stimulation (t=2.39; p=0.01), mainly due to enhancement in very low (t=2.23; p=0.02), low (t=2.16; p=0.02) and high (t=2.27; p=0.01) frequency ranges. There were no changes in the very high frequency range (Fig. 2A).

The LC stimulation of another 8 neurons induced a significant decrease of spectral average module sums in the whole frequency range only 1 min after cessation of stimulation (t=3.72; p=0.0003). Changes in the total frequency range were a consequence of the decrease of the average module sums in the low (t=3.21; p=0.001), high (t=3.45; p=0.0007; F=2.67, p=0.03)) and very high (4.02; p=0.0001) frequency ranges (Fig. 2B). There were no changes in the very low frequency range.

Discussion

Although this study has not elucidated the functional significance of the complex spike, as many studies have successfully done (Sato *et al*. 1992, Kitazawa *et al*. 1998), it showed that the interspike background activity in extracellularly recorded Purkinje neurons could be of functional significance. The effect of simple or complex spikes on interspike background activity of Purkinje neurons was tested immediately before and after their occurrence, in a sequence which corresponded to the time used for investigation of short-term modulation of simple spikes induced by a complex spike (Sato *et al*. 1992) or simple spike prediction of complex spike occurrence (Miall *et al*. 1998). In contrast to simple spikes, spontaneously occurring complex spikes induced changes in the spectra of interspike background

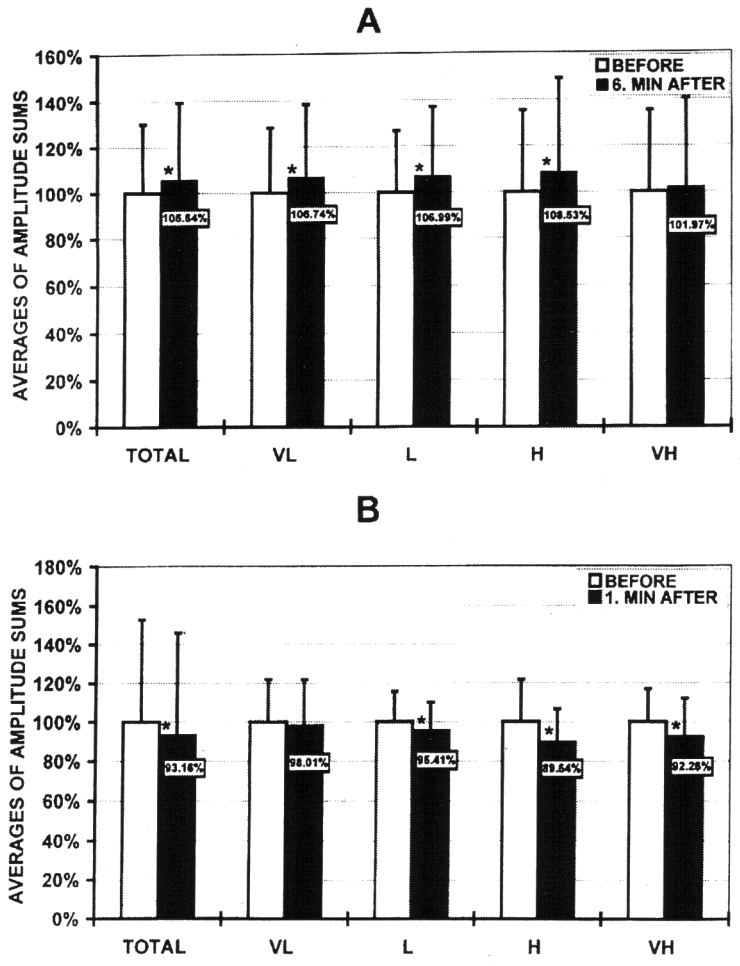

Fig. 2. *Spectral changes of interspike background activity caused by augmentation of excitatory and inhibitory input as a percentage of spontaneous values. Forty spectral averages of module sums from 8 Purkinje neurons per experimental group + CV (coefficient of variation), in the total (0-1500 Hz) and particular frequency ranges (VL= 0-249 Hz; L= 250-499 Hz; H=500-749 Hz; VH=750-1500 Hz) before and 6 min after the cessation of IO stimulation (A) and 1 min after the cessation of LC stimulation (B) (*p<0.05).*

activity. The changes of interspike background activity spectra could be the consequence of complex spike influences of single neurons, as well as of complex spike influences of neighboring Purkinje cells in the microzone around the recording site. Our results have also shown that, by recognition of all former excitatory synaptic and nonsynaptic events, background activity could foresee the expression of a complex spike. The discharge pattern of Purkinje cells was characterized by a significant increase of the total number of complex spikes only 1 min after cessation of IO stimulation, but there was a decrease of the total number of simple spikes only at 10 min (Ćulić *et al.* 1997). The IO has the highest known density of neuronal gap junctions within the central nervous system (De Zeeuw *et al*. 1995), and thus contains an anatomical substrate for generating simultaneous electrical discharges, which in turn could result in synchronous complex spike activity throughout zones of the cerebellar cortex. In most animals high levels of complex spike synchrony were limited to Purkinje cells separated mediolaterally by 250 μm or less (Lang *et al*. 1999). Therefore, a stronger excitatory input, through direct synaptic contact on a Purkinje cell, may change the spectral character of interspike background activity, because all complex spikes occur in the local microzone around our recording electrode. Due to particular recognition of excitatory inputs of different origin, as well as excitatory and inhibitory influences in general, interspike background activity could be an underestimated extracellularly recorded phenomenon. The recognition is faster in the case of inhibition than of excitation, probably due to plastic synaptic changes induced by IO stimulation. It is well known that their dendritic morphology enables cerebellar Purkinje neurons to provide optimal conditions for extracellular recording (Nadasdy *et al*. 1998). There are complex sources of extracellular current generation (Llinas and Nicholson 1971, Sprutson *et al*. 1995) and the action potentials in various brain structures may be affected by both intrinsic activity of the recording neuron and interneuron-mediated inhibition (Buzsaki *et al*. 1996). Buzsaki's recording procedure in the case of cerebellar Purkinje neurons and

our proposed direct spectral analysis of the extracellularly recorded signals, particularly the interspike background activity, could be a possibility of elucidating the nature and origin of interspike background activity. For the moment, our study only suggests that, besides the ability of neurons to transmit information through spike outputs (Buzsaki *et al*. 1996), the synaptic and nonsynaptic background activity could be of some physiological significance.

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