Realtime wavelet-based clustering for a 64 channel multisite recording system

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Abstract

We report on the practical use and results of spike detection and semi-automated clustering algorithms based on the discrete wavelet transform implemented on a digital signal processor. In vivo extracellular recordings were conducted in rat visual cortex with a fully digital data acquisiton system and new batch fabricated 64-site microelectrodes. We utilized two commercially available multichannel amplifier chains using the same probe and data acquisition system. All signals are recorded with a resolution of 16 bit and piped into our new online spike clustering tools, based on features extracted from the spikes by wavelet coefficients.

Introduction

Modern electrophysiology is strongly turning towards acquisition and processing of signals of big neuronal populations and large tissue samples with high signal quality [1-4, 5, Biella, 2002 #726]. This can be done parallel to the brains lamination by micro-electrode matrizes (which record from many single shafted metall wire- [6, 7] or silicon spike-tips [8]) or perpendicular to the lamination by fork-shaped silicon probes each of their tines carrying many recording sites. In any case, recording from many sites becomes more and more a standard procedures, which obviously creates an increasing need for machine-supported analysis, simply due to the fact of otherwise overwhelming amounts of raw data. Within the European Union financed VSAMUEL project, we develop not only new, minimal traumatizing silicon recording probes, but a whole new complete measurement chain, from signal to spike train [9-11]. In this work, we report on the first recordings with ready-to-use 64 site probes from rat visual system in vivo and the advanced signal processing tools to decompose the raw signals into population spike trains (spike detection on single channels). In addition, a semi-automated realtime clustering technique is demonstrated, based on features extracted from the individual spikes wavelet coefficients [12].

Materials and Methods

Surgical Procedure

The results presented here are based on recordings in the primary visual cortex (area 17) of rats (Brown Norway,300 –380 g). They were anesthetized with an i.m. injection of ketamin/xylacin/chlorpromacin (10mg/0,4mg/1mg / 100g b.w.) during surgical preparation and maintained with a nitrous oxide/oxygen (30/70%)–isoflurane (0.5%)gas mixture during the recording session. During surgery animals were placed in a stereotaxic apparatus, body temperature was kept constant,and the heart rate was monitored continuously. The cornea was protected with a non-refractive contact lens throughout the experiment. The scalp was removed, and a small (2×2 mm)bone window above the left visual cortex was drilled (centered at AP =+1 and =3,25 mm from lambda),the dura was reflected and, after electrode positioning, the cortical surface covered with 3%Agar in Ringers solution to prevent drying of the brain and decrease pulsation. All procedures used in this study were performed in accordance with the guidelines for the welfare of experimental animals issued by the Federal Government of Germany, approved by local authorities and conformed to the guidelines of the National Institutes of Health for the care and use of laboratory animals.

Response properties and appropriate receptive field (RF)boundaries of cortical cells were determined qualitatively with visual stimuli generated by a hand-held pentoscope and projected on a tangent white screen. Then RFs were centered on a monitor (EIZO FlexScan F87) positioned 57 cm from the animal.S timuli consisted of whole screen black and white gratings, moving with constant velocity (5 –20/s) and spatial frequency (0.08 –0.6 cycl deg).Background illumination was kept below 1 cd/m^2, and stimulus intensities ranged from 7 to 10 cd/m^2.Each experiment consisted of several blocks of trials in which 18 stimuli with particular moving direction were presented in a pseudo-random order.

Silicon Probes

Recordings were performed with two types of micro-machined multi-site recording probes, now commercially available from the ACREO AB, Sweden. Both probes consist of 4 shafts of 5mm length carrying 16 electrodes each on the silicon substrate with silicon-nitride passivation. The cross section of their 2mm (E6), or 1,2mm (E4) long "sensitive" area (i.e. having sites exposed) is 38µm×30µmm, thus having a very high "site per displaced volume ratio". One micro-machined probe (E6) features 64 co-planar electrodes with a distance of 100µm between centers along a tine and 400µm between tines. The other probe (E4) has the same amount of sites, but displays stereotrodic arranged sites, i.e. 2 sites with a center/center distance of 30µm are placed in 100µm steps along the tines (Fig. 1). Electrodes are 100µm^2 rectangles of sputtered platinum,with typical impedances of 2-3MΩ at 1 kHz. The probe tines' outlines are shaped by a plasma etch and the fabrication process is described in detail elswhere [13].

Data Acquisition

Signals were amplified either by using the 64 channel programmable gain amplifier and headstage from Uwe Thomas Recoding GmbH (Giessen, Germany), designed to be used with those silicon probes, or by painfully adapting the flex-board connector of the probes to another commercial

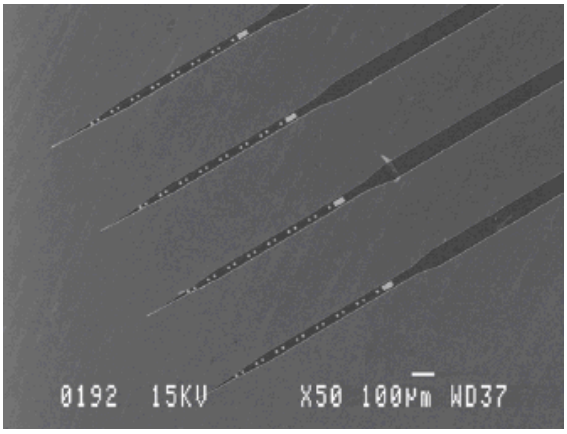

Fig. 1: Electron micrograph of a 64 site silicon probe in stereotrode arrangement.

The subsequent data acquisition system is based on stackeable, commercially available digital signal processing boards (M67, Innovative Integration, Thousand Oaks, CA) in a standard Windows PC, utilizing a wavelet transformation framework described in detail elsewhere [14]. Each boards acquires signals from 32 channels via one Sigma-Delta A/D converter per channel without any multiplexing at a rate up to 50 kHz per channel. The A/D converters are connected to the oustide world with a standard 68-pole SCSI-socket and are thus easily connected with the appropriate connector to all multi-channel amplification systems able to provide ±10V signal output.

Results

Results of Sorting Algorithm

As was mentioned before [9, 12], there are several advantages to decompose neural signals into by the discrete wavelet transform. We implemented this approach by a fast online framework and perform spike detection and clustering on the wavelet coefficients in addition to standard signal processing.

We are still investigating the question of the optimal and still most practical spike detector with artificial, but realistically simulated extracellular signals [15, 16]. In the meantime, the seemingly most reliable spike detection was performed with a simple threshold passing detector either on the original signal or on the results of a non-linear energy operator (NEO [17]).(Fig. 2)

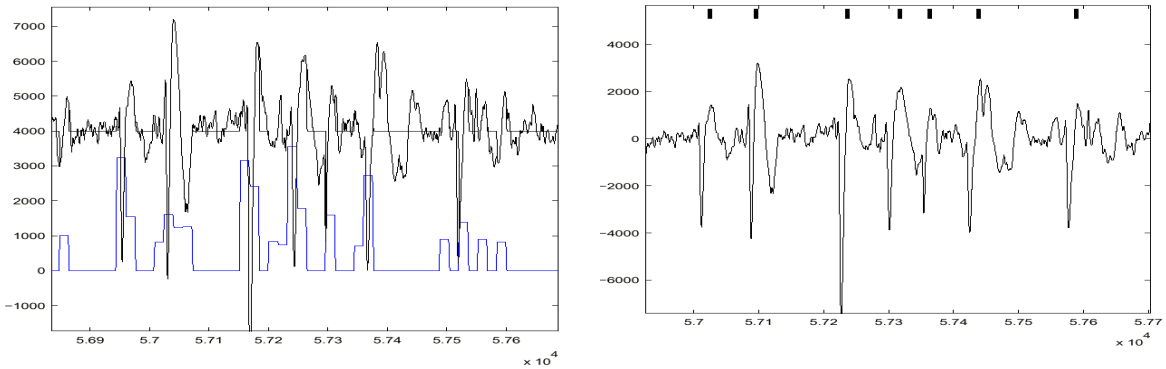

Fig 2. Example section of 168 msec length. Left: NEO coefficients (bottom) for example (top). Right: Series of the spikes detected by thresholding the NEO output.

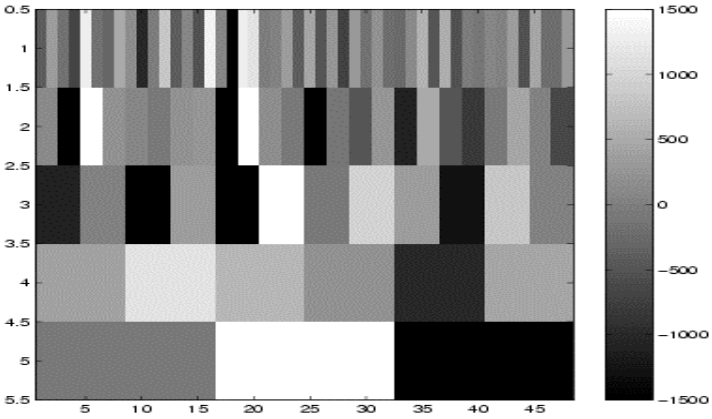

Fig. 3: Scalogram of the spike of Fig. 2 right. The x-axis depicts sample points within the original signal and the y-axis gives decomposition levels, strongly related to frequency bands. Gray-level codes for energy contained in that particular sampling point.

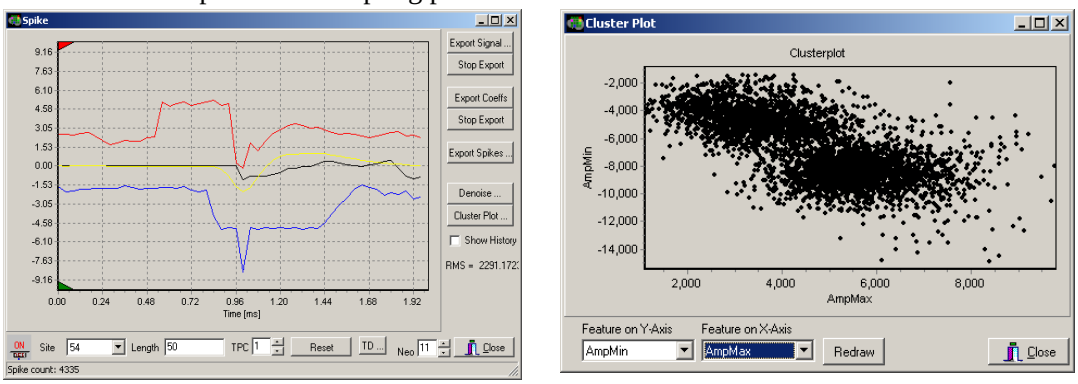

Fig. 4: left: History or overlay plot of detected spikes. Right: Plot of our general use cluster display. Display was performed here on the classical features of signal amplitude minima and maxima values. Instead, other features like our newly proposed peak-to-peak amplitude within one decomposition level are possible as well.

For further real-time clustering, we calculate within all decomposition levels for each pre-detected spike snippet the difference between the levels' biggest and smallest coefficients $\boldsymbol{pp_i}$, (compare to Fig. 3). We find for each level i and snippet a characteristic value $\boldsymbol{pp_i}$, which serves as feature value in a cluster plot (Fig. 5).

At the time of this writing we did not implement an automated cluster cutting scheme into the realtime running software. Instead we gave the user the choice to mark on the appropriate plot visually visible clusters by an adjustable ellipse, thus choosing from the whole set of spikes those apparently belonging to one firing unit (Fig. 6).

Selected spikes are then treated separately, displayed (Fig. 7), stored with their spike patterns and spike times and thus identifiying an individual cell with its own spike train.

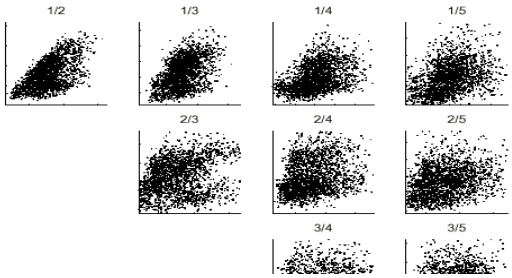

Fig. 5: Cluster plots derived by displaying the characteristic differences $\boldsymbol{pp_i}$, in each levels maxima and minima of a particular spike against each other.

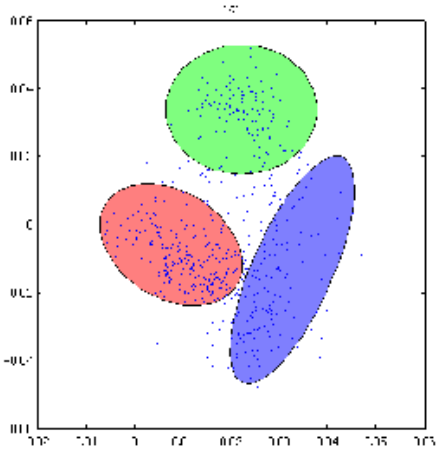

Fig. 6: Screen shot of three visually detected and marked spike clusters in a display of pp_2,versus pp_3.

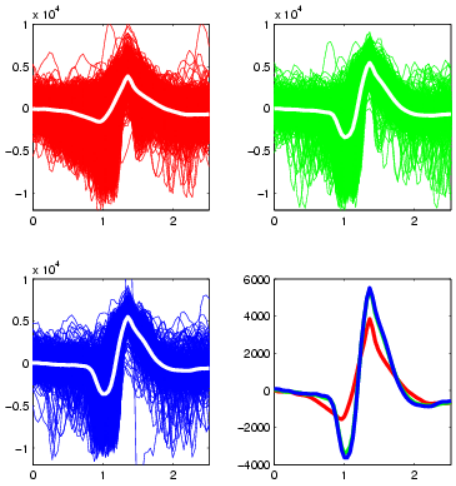

Fig. 7: Averaged display of spikes found marked in Fig. 6. It is clearly visible, that "green" and "blue" do not belong to different populations

Results of Visual Stimulation

Our experiments demonstrate the feasibility of multi-site recordings in the rat visual cortex with micro-machined, high site number recording probes. Importantly, spike activity was successfully recorded throughout cortical depth, and visually triggered.

Discussion

The new multichannel recording system and the multisite probes were succesfully integrated in an existing recording setup and may thus be a welcomed addition to long standing procedures. It performs spike detection and some type of semi-automated clustering on all channels in realtime, i.e. while the experiment is performed and the whole raw data stream is stored to disk. Experimenters currently experienced in single-wire recordings will have to adapt to not being able to hunt for a single cell with the tip, but instead have to take "what they get" with a high number of electrodes instead.

Acknowledgments

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