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Improvement of Nociceptive Spike Clusterization with Shape Approximation

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Abstract Cluster spike analysis is widely used for studies of neuronal activity when electrical signals are sorted out and grouped according to the different shapes. We recently applied this method to sort out the nociceptive spikes in the trigeminal nerve implicated in generation of migraine pain. However, the electrical noise leading to less accuracy of calculated spike parameters often hinder the correct sorting of nerve signals. In this study, in order to improve the accuracy of calculations, we explored the prior approximation of spike shapes before applying clusterization. The prior fitting of spike shapes allowed us to extract signal parameters much more precisely and detect the strongly increased number of spike clusters which is close to the expected number of fibers in the trigeminal nerve. Prior approximation improved cluster analysis outcomes and, importantly, revealed new clusters that demonstrated the different functional properties, suggesting that their function was previously hidden within the multiple firing.

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1 Introduction

Analysis of spikes recorded by different electrophysiological techniques is the most reliable current approach to evaluate the function of the neurons in the central and peripheral nervous system [1–4]. Given a large number of synapses and complicated morphology of neurons, spiking activity is typically very heterogeneous, thus, requiring advanced methods for separation of multiple spikes. One of the commonly used approaches for obtaining physiologically relevant information on the neuronal activity is the clustering of spikes [3, 5]. Thus, the cluster spike analysis based on signal grouping according to the amplitude and the shape of spikes is commonly used to identify spikes originating from different cells. Basically, there are various ways for spike features extraction: principal component analysis [5, 6], wavelet analysis [7, 8], or the direct calculation of the parameters [9].

To improve the quality of registration and to reduce the contribution of the noise to spike numerical description, different types of filtration, both analog and digital, are commonly used. However, all types of filtration, especially in the case of low signal/noise ratio, can modify the original signal, thus affecting physiological conclusions.

Recently, we applied clusterization method to sort out the nociceptive spikes generated in the peripheral branches of the trigeminal nerve in meningeal tissues [9]. This study provided a novel information on the neurochemical mechanisms of nociception in trigeminal nerve endings implicated in generation of migraine pain.

In the above study, we used the recordings with suction electrode [10] resulting in relatively low amplitude signals.

Therefore, in the current study, we decided to employ the prior approximation of the recorded spikes in order to overcome the low single/noise ratio and more accurately calculate the amplitude and the time course of nociceptive signals. After prior approximation, we performed spike clustering using KlustaKwik 1.7 program [11]. This approach strongly increased the number and quality factors of detected clusters and allowed us to reveal new properties of trigeminal nociceptors.

2 Methods

Experimental data used for prior approximation method development were obtained from the study of capsaicin-induced neuronal activity in rat meningeal nerve fibers. In short, experiments were performed on male P40–42 Wistar rats at room temperature (22–24 °C). The skull was thoroughly cleaned from the surrounding tissues, divided into two halves and the brain was carefully removed. The resulting hemi-skull was continuously superfused in oxygenated (95% O2/5% CO2) Krebs solution composed of (in mM) NaCl 115, KCl 3, CaCl2 2, MgCl2 1, NaH2PO4 1, NaHCO3 25, and glucose 11; pH = 7.3. Immediately before the experiment, the peripheral large branch of the trigeminal nerve in meninges was removed from the surrounding dura mater and placed in a recording glass electrode. Capsaicin (1 μM, diluted in DMSO, the final concentration of latter 0.01% which did not affect firing) was applied to the area of divergence of the medial meningeal artery (MMA) in the dura mater.

The choice of capsaicin treatment was due to remarkable pro-nociceptive effect of capsaicin, largely increasing the number of spikes in trigeminal nerve fibers (Fig. 1a). The total number of spikes in these experiments ranged between 1000 and 9000, which provided essential dataset for reliable calculations.

Custom written program in MATLAB was used offline to detect spikes in digitized electrical recordings [12]. Suprathreshold, i.e., greater than five standard deviations (SD) of a baseline recording, electrical activity was considered as a spike event. Time windows of 6 ms with 2 ms before and 4 ms after positive peak of the spike were pulled out for further analyses as described by us elsewhere [12]. Spikes collected were normalized by amplitude to SD of each recording base line to account for differences in recording conditions.

The typical experimental spike has the positive and negative phases (Fig. 1b). As positive and negative phases of the spike are based on different ionic mechanisms, we decided to approximate each of these phases separately (Fig. 1d). To improve the quality of approximation, the end parts of the positive phase were overlapped with initial parts of negative phases. In Fig. 1d, this overlapping range is indicated by black

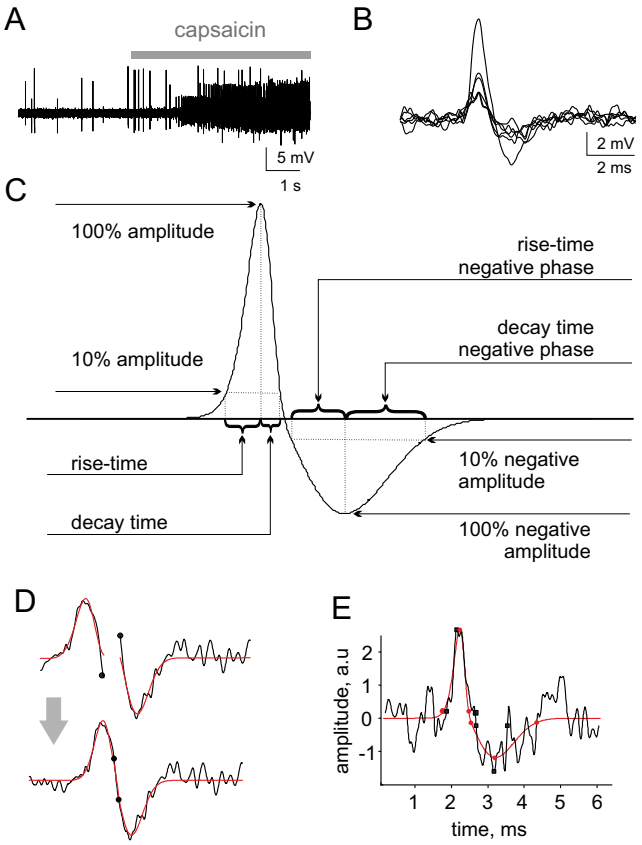

Fig. 1 Spike detection and shape approximation. **a** Original trace of spontaneous multiple unit activity obtained from the peripheral trigeminal nerve stimulated with 1 μM capsaicin. **b** Example of variable amplitudes and shapes of spikes detected in trigeminal nerve. **c** Scheme showing the calculation of action potential parameters such as amplitudes of the positive and negative peaks as well as the rise time and decay time. **d** Overlapping positive and negative segments of the action potential used for approximation after separation (up) and after reconstruction (bottom). **e** Example of approximation (*red*) of the original spike (*black*). Filled *black* and *red circles* indicate key points of spike calculated from original and approximated shapes, respectively

dots. As a result, we obtained a complete signal with a smooth transition from the positive to negative phase.

To approximate the time course of spikes we used the Weibull function, [13] as it allows to scale the signal on the axes and to choose the final waveform. This distribution is represented by the following formula:

$$f(t) = a*\left(\frac{t}{b}\right)^{(c-1)*} e^{-\left(\frac{t}{b}\right)^{c}}$$

where a is a coefficient for scaling in the vertical axis, b is for time scaling, and c is the coefficient to set the shape of the curve.

Fitting of positive and negative phases to Weibull function was done by varying above the coefficients using MATLAB function - nlinfit (X, Y, modelfun, beta0). In brief, 6 ms recording representing a spike was put in X vector, modelfun

was our Weibull function, beta0 vector was used to set initial values for a, b, and c coefficients. As a result, this function returned Y vector containing estimated a, b, and c coefficients that were finally used to represent approximated spike.

Figure 1e shows an example of experimental (black line) and calculated signal after approximation (red line). Black dots in the curves indicate the key points of original signal (peaks, onsets and offsets of positive and negative phases). Red dots mark the same for calculated curve.

Main spike parameters, which were calculated on both raw recordings and approximated spikes time courses in the current study, are shown in the Fig. 1c. The following parameters were calculated: positive and negative amplitude, area of each phase, rise time (between 10 and 100% of positive amplitude), and decay time (from 100 to 10% of positive amplitude) (Fig. 1c).

These parameters were entered to the KlustaKwik to analyze clustering of both raw and approximated spikes.

All calculations were performed by using the MATLAB software (MathWorks, USA) with the KlustaKwik tool box. Statistical analysis was performed with two tailed t test and results were considered significantly different when $p < 0.05$.

3 Results

The efficiency of calculations with spike approximation was obvious even with visual inspection, as the density of spikes in clusters was much more compact (Fig. 2a–d). Thus, the density of the cluster calculated without approximation was much lower (Fig. 2a) than of cluster obtained after approximation (Fig. 2b).

Even more interesting was the finding that, after approximation, the previously homogenous large size cluster (Fig. 2c) was divided into two clearly distinguishable smaller clusters (Fig. 2d). Remarkably, in this case, the histograms of corresponding spike parameters (inset to Fig. 2d) indicated two non-overlapping Gaussian-like distributions.

To assess the effectiveness of spike approximation on the quality of clustering, we constructed the temporal distribution of parameters of spikes, which were grouped into one cluster. To this end, we compared the time course of firing frequency in control (Fig. 2e) with respective new clusters obtained after approximation (Fig. 2f). Interestingly, the newly identified clusters demonstrated significantly a different time course of firing. In one case (upper trace of Fig. 2f) the firing was

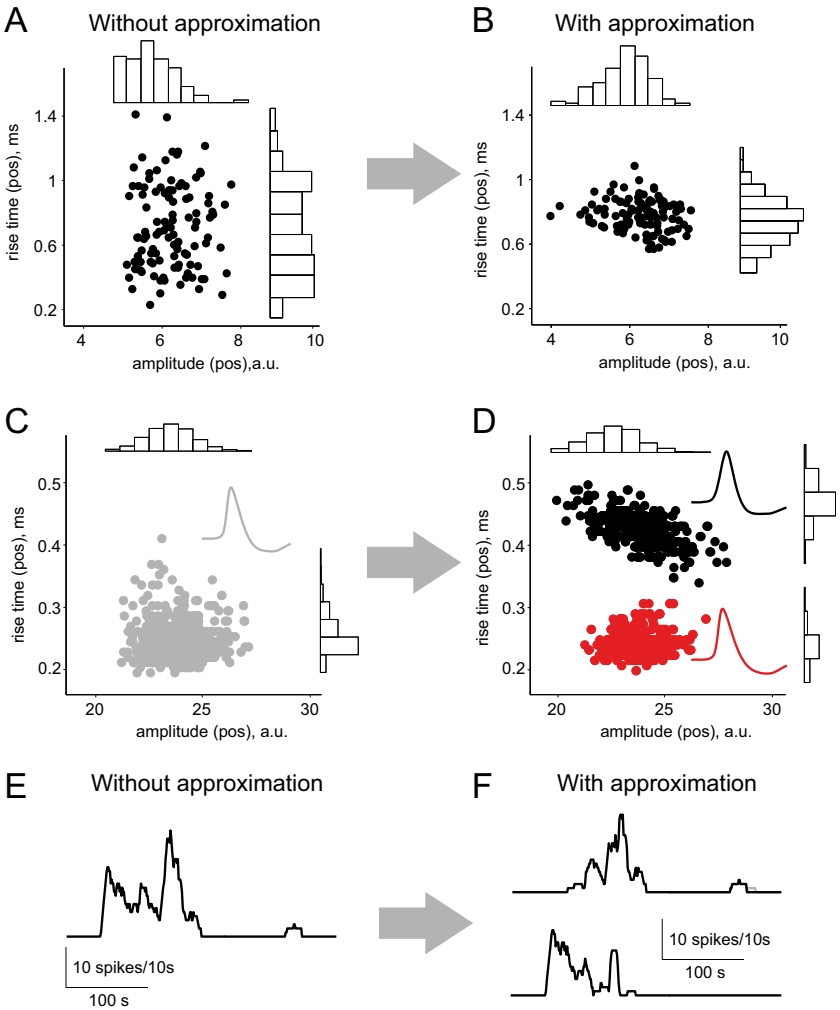

Fig. 2 Effect of approximation on spikes distribution in parameter space. **a** and **b** show data of one experiment where spike parameters were calculated before (**a**) and after approximation (**b**). Each *black point* on graphs corresponds to a single action potential. The distribution of the positive rise times versus positive amplitude after approximation is shown. **c** and **d** obtained from the other experiment to illustrate the separation of a single cluster (*presented as a group of circles*) into two non-overlapping groups after approximation. **e** and **f** show the effect of approximation on the time course of capsaicin-induced firing. Notice the slowly growing responses in the upper trace and almost instant response in the lower trace after approximation

growing slowly, possibly reflecting a secondary effect, whereas in the other case (bottom trace), there was a very steep rise in activity suggesting an immediate opening of TRPV1 channels. Thus, the prior approximation revealed previously hidden different temporal properties of the neurochemical responses in the trigeminal nerve.

For numerical evaluation of effectiveness of our approach, we calculated the number of clusters obtained with and without approximation. Figure 3 shows an example of the experiment when the previous method without approximation detected 20 clusters from 7061 spikes (Fig. 3a). However, after approximation, we identified 54 clusters. Notably, in the latter case (Fig. 3b), clusters were occupying a larger space and were presented by better separated groups (multiple individual clusters). Improved separation is confirmed by Mahalanobis distance which indicates the "remoteness" of clusters from each other, assuming the higher distance, indicating the better clusterization. We found that the Mahalanobis distance (calculated as mean distance between each clusters and averaged across all experiments) was significantly higher for approximated spikes (24.1 ± 2.5) than for original spikes (16.4 ± 1.4, $n = 17$, $p < 0.05$ with t test, Fig. 3d).

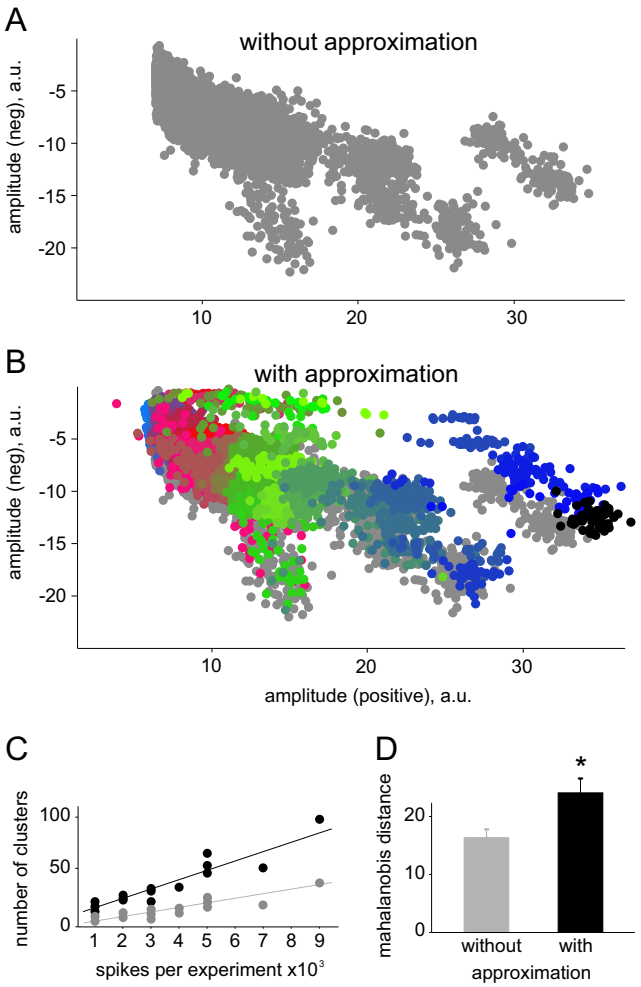

Fig. 3 Approximation increased number of detected clusters. **a** and **b** show results obtained from an individual experiment to illustrate the full set of 20 clusters before (**a**) and 54 clusters after approximation (**b**). Each point corresponds to a single action potential. Clusters in **b** are overlapping with gray points from **a** to show their deviation. **c** Number of clusters found using conventional parameter calculation (*gray*) and after approximation (*black*) versus number of detected action potentials. **d** Average Mahalanobis distance of cluster obtained before (*gray bar*) and after approximation (*black bar*). $n = 17$, $^*p < 0.05$ with t test

4 Discussion

The most important advance of our study is improved detection of clusters of spikes recorded from the meningeal trigeminal nerve resulting in better description of physiological properties of peripheral nociceptive fibers critical for generation of migraine pain.

The spike approximation applied in this study to analyze the nociceptive firing in trigeminal nerve during physiologically relevant stimulation, revealed an essential deviation of experimental spikes from the idealized shape mainly due to noise contribution. Therefore, a more careful spike inspection is most important for low amplitude spikes with low signal/noise ratio [3, 14–16]. More specifically, for small signals, the detection of the amplitude from the peak was largely dependent on the presence of contaminating noise on the peak. In addition, the resulting rise time of the spike analyzed without approximation could vary by several folds. Consistent with this, approximation which largely standardized (smoothed) the rise time was most effective in the cases with small fast spikes resulting in detection of more clusters. Interestingly, the total number of clusters detected with the method of prior approximation approached 100, which is close to the expected number (120–150) of fibers in the trigeminal nerve detected after specific labeling [10].

The validation of our approach was performed by comparing the distribution of rise-time parameters before and after approximation. The Gaussian type of distribution in the latter case indicated more reliable approach for spike clusterization.

By analyzing the distribution of spikes in clusters obtained with and without approximation, we found that in most cases, the single cluster was divided into several new clearly distinguishable clusters. Notably, this transformation may change, in some cases, the frequency of firing in the new cluster and can modify even the temporal profile of response. Thus, most interesting was that new clusters often demonstrated new temporal patterns of activity. Previously, we found that the trigeminal nerve in meninges contains about 65% of capsaicin-sensitive TRPV1 positive clusters [9]. This number in the current study was 68% in control and 72% after approximation that exceeds by ~2-times an expected fraction of TRPV1 positive trigeminal neurons we previously counted in cell culture [17]. Since we also found here that activity in new clusters could develop either instantly or slowly, the latter could be due to secondary indirect activation of nearby fibers by endogenous agents released from the fast responder. This is

consistent with our previous observation that the activation of the trigeminal nerve could be presented by "early" and "late" responders [9]. The late responders could be activated via release of endogenous active molecules such as extracellular ATP which can provide massive activation of trigeminal nerve terminals [18]. Moreover, the approximation of spikes resulting in a larger number of clusters could provide a more precise fractional analysis and a better link between clusters and single fibers in the trigeminal nerve branches [9, 12, 19].

5 Conclusion

In conclusion, we suggest that prior spike approximation is an important component of the clustering analysis of spiking activity especially when the contribution of the negative factors such as the noise, is essential. Approximation can provide more detailed analysis of spiking activity both in the peripheral and central nervous system, allowing detection of neuronal functional properties which otherwise are hidden in multiple firing activity.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights Experiments were performed in accordance with the European Community Council Directive of September 22, 2010 (2010/63/EEC) for animal experiments and all animal-use protocols were approved by Kazan Federal University on the use of laboratory animals (ethical approval by the Institutional Animal Care and Use Committee of Kazan State Medical University N9–2013).

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