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Long-Term Recording of Single Neurons and Criteria for Assessment

L. N. Vasil'eva,[1,3] A. M. Badakva,[2] N. V. Miller,[2] L. N. Zobova,[2] V. Yu. Roshchin,[1] and I. V. Bondar'[1]

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Stable recording of the activity of single neurons in the brains of vertebrates can provide investigators with information on the processes underlying the plasticity of the nervous system. In practical terms, recording of the activity of individual neurons over long periods of time is relevant to the development of invasive brain-computer interfaces. We propose criteria for identifying neurons whose activity is present on traces for more than one day. Classification exclusively on the basis of action potential shape is extremely unreliable. Addition of supplementary comparison parameters to the classification, such as the pattern of the distribution of interspike intervals or the characteristics of this distribution, significantly decreases the probability of classification errors. Application of criteria to neurophysiological data allowed us to identify 82 neurons in our experimental data whose activity was present on traces lasting two days or more, and one neuron could be recorded for 94 days.

Keywords: chronic recording of single-neuron activity, brain-computer interfaces, primary motor cortex in monkeys, criteria for assessment of the stability of recording extracellular activity.

Plasticity is an intrinsic property of the nervous system, providing organisms with the ability to adapt to changing environmental conditions. In response to external factors, single neurons can alter their discharge characteristics (frequency and time pattern), which may correlate with adaptive changes in the animal's behavior [Op de Beeck et al., 2007]. As a rule, studies of the mechanisms of plasticity at the level of single nerve cells are limited to periods of a few hours [Op de Beeck and Baker, 2010]. Studies of the properties of single neurons for longer periods of time – days, weeks, months, and even years – can provide investigators with unique information on the mechanisms of brain plasticity. Research technologies are now available for prolonged observation of the activity of single neurons, allowing the lives of individual cells to be followed for several weeks or even months [Fraser and Schwartz, 2012; Jackson and Fetz, 2007; Tolias et al., 2007]. These studies require the application of objective criteria for sorting activity recorded at different times and assigning it to one neuron or to different neurons. The success of sorting requires identification of cell discharge parameters which are stable over time.

Development of criteria for assessing the stability of recordings on the basis of the spontaneous activity of individual neurons is the most preferred approach. Naturally, the first criteria for assessment of the ability of neuron recordings were based on action potential (AP) shape [Jackson and Fetz, 2007]. The main assessment criterion for stability was the linear coefficient of the correlation between AP shapes. This approach was criticized because of the high proportion of erroneous classifications [Fraser and Schwartz, 2012] arising on identification of neuron activity recorded on different days. The probability of ascribing the AP of different neurons to one cell and vice versa – ascribing the AP of one cell recorded on different days to different neu-

[1] Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia; e-mail: bondar@ihna.ru.
[2] Institute of Medical-Biological Problems, Russian Academy of Sciences, Moscow, Russia.
[3] Pirogov Russian National Medical Research University, Moscow, Russia.

rons – is high. In particular, there is a need to decrease the probability of erroneous classifications by supplementing comparison of AP shape with other characteristics of spontaneous neuron discharges.

Additional characteristics for comparison may include, for example, the shape of interspike interval histograms (IIH). Criteria for comparing IIH may not be strong enough because of the morphological features of brain structures in which recordings were made. In the case of cortical areas with column-based organization, the activity of cells in one column may be synchronized, leading to increases in the probability that similar IIH would appear and, as a result, erroneous classifications [Tolias et al., 2007]. Tolias et al. recorded neuron activity using tetrodes chronically implanted in the primary visual cortex and sought to identify neurons' "electrophysiological signatures" which would allow their activity to be followed over long periods. Stability was evaluated using the distance between AP characteristics in a 28-dimensional coordinate system. Use of this approach showed that individual neurons could be recorded chronically for days and weeks.

Other supplementary classification parameters include the nature of a neuron's discharges during the peristimulus period [Bondar et al., 2009]. Correlation coefficients for relationships between peristimulus histograms were used to determine whether activity recorded on different days belonged to one neuron or different neurons. This criterion cannot be used for recordings including only spontaneous neuron activity.

A further classifier for stable neurons was developed recently, this including four different neuron discharge parameters: AP shape, mean spontaneous spike frequency, cross-correlograms with the activity of other neurons, and IIH [Fraser and Schwartz, 2012]. These were used to calculate a similarity parameter for neurons. If this was high enough, AP were assigned to a single neuron. Some neurons could be recorded for more than 100 days. Following the activity of individual neurons over prolonged periods of time allows evaluation of the rearrangements underlying modifications to animals' behavior.

The aims of the present work were to use the characteristics of spontaneous discharges to develop criteria for assessing the stability of recording of single neurons and to evaluate the potential for prolonged observation of the activity of individual nerve cells in the primary motor cortex using chronically implanted microelectrodes.

Methods

Recording of neurophysiological data. Experiments were performed on two monkeys. All animal manipulations were performed in compliance with the requirements of European Community Directives (86/609/EEC) regarding the use of animals for experimental studies.

Neurophysiological signals were recorded using bundles of nichrome microwires of diameter 12 μm. Bundles of microwires were implanted into the brain tissue via guide cannulas previously implanted into the animals' skulls [Miller, 2008]. Detailed descriptions of the bundle implantation procedures were presented in our previous report [Bondar' et al., 2014].

Recording quality was followed by making regular 5-min recordings of neuron activity using the 15 microwires of the bundle. Neuron signals were recorded using an apparatus from Neirobiolab (Russia). The sampling frequency for recording signals was 25 kHz and frequency filtration was over the range 0–10 kHz.

Sorting of action potentials. Recorded neurophysiological signals were processed using the program Spike2 7.02 (Cambridge Electronic Design, UK). Native data were filtered in the range 300 Hz to 10 kHz, after which amplitude thresholds in continuous neuron signals were used to extract AP. The threshold amplitude for identification of AP was essentially twice the standard deviation of the mean amplitude of background noise. Extracted AP were finally grouped into clusters using principal components analysis. The cleanness of AP discrimination was assessed in terms of interspike interval histogram shape: the presence of a clear refractory period confirmed successful extraction of the activity of an individual neuron.

AP-containing trace segments of duration 2.1 msec were stored in Matlab format and further data processing was run in programs created in Matlab 7.6. These programs averaged AP, calculated correlation coefficients for relationships between AP shapes, and also ran all the calculations described below.

Development of stability criteria. We elected to develop stability criteria for neuron activity recordings to allow determination of whether activity recorded on different days should be assigned to one or several neurons.

In order to identify whether neurons could be discriminated on the basis of AP shapes, we plotted the distribution of correlation coefficients for relationships between mean AP obtained from a single cluster. An analogous distribution was plotted for correlation coefficients for relationships between traces from neurons known to be different (Fig. 1). Analysis of the distribution of correlation coefficients for AP shapes, IIH shapes, and the distribution of the distances between vectors was used to determine critical values for similarity, and the probability of classification error was calculated.

Results

Recording stability criteria. At the initial stage, we evaluated the reliability of classifying neuron activity using comparison of AP shape. AP from different neurons are known to have characteristic features, so it seems logical to use this neuron activity parameter. The measure of AP similarity was the correlation coefficient. In the situation of a single neuron, the correlation coefficient approaches unity.

In order to understand whether neurons can be discriminated on the basis of correlation coefficients of the relationships between AP requires values for correlation coefficients for relationships between AP from neurons known to

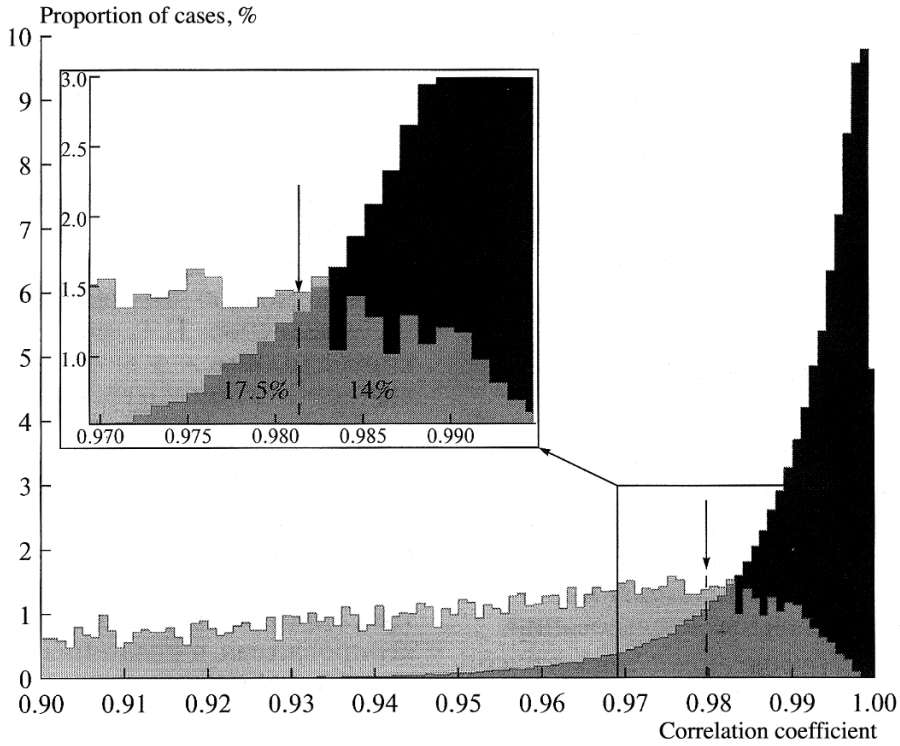

Fig. 1. Distribution of correlation coefficients for relationships between action potentials known to be different (recorded in different animals; gray histogram) and action potentials known to be identical (from a single cluster of action potentials; black histogram). The ordinate shows the proportion of casers (%). The arrow shows the critical value of the correlation coefficient – 0.98. insert: the histogram overlap intersection point, showing the proportion of different action potentials whose correlation coefficients were greater than the critical value (false positives) and the proportion of identical action potentials for which correlation coefficients were less than the critical value (false negatives).

be single and neurons known to be different. In the present study, AP from neurons known to be different were AP recorded in different animals. AP known to be from single neurons were those present in a recording channel on the same day and assigned to one cluster by the principal components method.

The reference group of different AP consisted of the activity of neurons obtained by recording signals in different monkeys. Figure 1 shows the distributions for the two comparisons. The mean correlation coefficient for AP from a single neuron was 0.988 ± 0.022, while that for different neurons was 0.575 ± 0.610. Comparison of the sets using the Kolmogorov–Smirnov test showed them to be significantly different ($p < 0.001$). Thus, overall, comparison of AP using correlation coefficients allows quite precise determination of the extent of similarity between AP. We felt that it would be possible to identify a threshold value for the coefficient to evaluate cell stability.

Figure 1 (insert) shows the overlap point of the two distributions of correlation coefficients: one for cells known to be different and one for recordings from a known single cell. The critical value for the correlation coefficient for AP shapes was taken as 0.98, as the envelopes of the two distributions intersected at this point. In this case, the probabilities of false positive and negative identifications were 14% and 17.5%, respectively. Thus, AP shape alone was not sufficient for reliable classification of neuron activity. Analogous considerations were used for correlation coefficients for IIH and the distance between the vectors defined by the characteristics of the interspike interval distribution (mean, median, standard deviation, and coefficient of asymmetry).

IIH were plotted and distances between the characteristics of the distributions of interspike intervals were calculated by dividing each trace into two parts, each of which was analyzed. Further comparisons were performed for two situations: for the activity of a single neuron on one electrode and for neuron traces from different electrodes. The mean correlation coefficient between IIH for identical neurons was 0.783 ± 0.173, while that for different cells was 0.377 ± 0.335. Comparison of data using the Kolmogorov–Smirnov test identified statistically significant differences ($p < 0.001$). Sets for distances between vectors consisting of IIH characteristics were also statistically significantly different in terms of mean values, medians, standard deviations, and coefficients of asymmetry. The mean value and dispersion for identical neurons were 0.508 ± 0.383, compared with 0.169 ± 0.160 for different neurons. The distribution data were used to calculate the proportion of erroneous classifica-

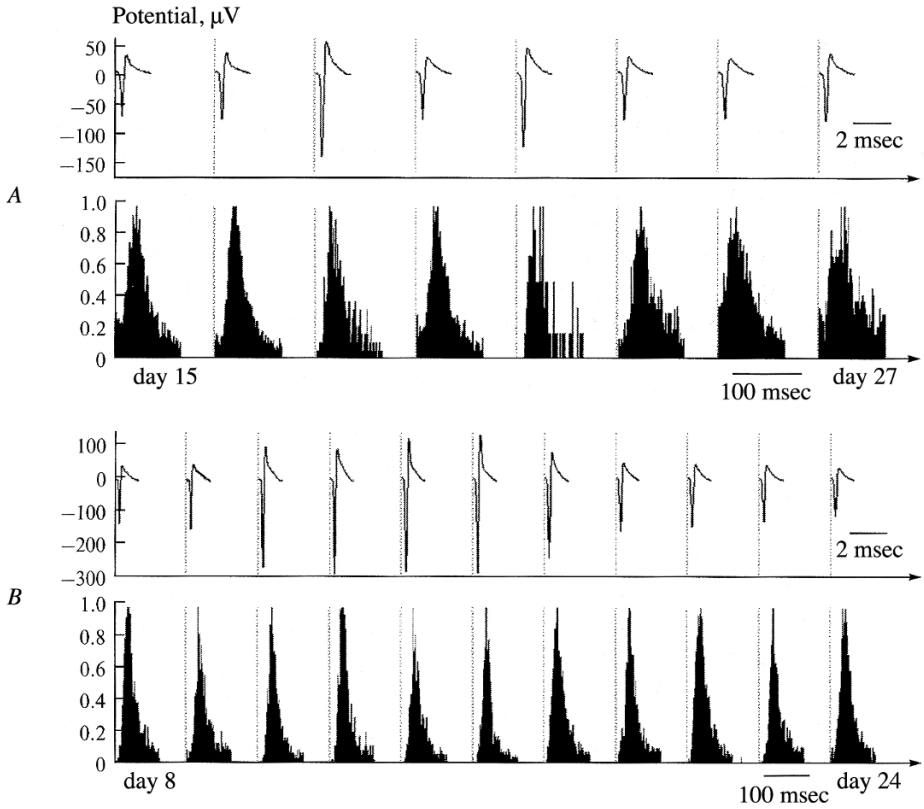

Fig. 2. Examples of long-term recording of activity from a single neuron. Action potentials and interspike interval histograms on different recording days. *A*) Action potentials and interspike interval histograms for neuron No. 22 (12 days of stable recording); *B*) action potentials and interspike interval histograms for neuron No. 42 (17 days of stable recording).

tions using 0.7 as the threshold value for correlation coefficients and 0.3 for distances. The proportion of false positive identifications based on correlation coefficients for relationships between IIH was 14.1%, compared with 25.3% for false negative identifications. Classification performed on the basis of distances between vectors of IIH characteristics had a proportion of false negative identifications of 14.4% and a proportion of false negative identifications of 40.4%. As in the case of correlation coefficients for relationships between AP shapes, the error proportions were quite high.

With the aim of increasing the power of the test, we used a classification based on two criteria. In this case, only those neurons for which two similarity measures were identical were taken as identical. In the case of classification based on correlation coefficients for the relationship between AP shapes and IIH shapes, the probability of false negative classifications was 0.361%, compared with 3.39% for false positive classifications. In the case of classification based on correlation coefficients for the relationship between AP and IIH parameter vectors, the probability of false negative classifications was 0.194%, compared with 7.39% for false positive classifications.

Thus, only simultaneous use of several characteristics of spontaneous neuron discharges provided for a decrease in the number of erroneous classifications. It is interesting to note that this analysis, by comparison, does not require accumulation of a large amount of experimental data. We believe that the variant involving a trace of spontaneous activity lasting 3–5 min is entirely adequate to the purpose.

The possibility of long-term observation of the activity of individual neurons in the motor cortex. Examples of stable recordings are shown in Fig. 2. In one case, the signal from an individual neuron could be recorded over 13 days; another could be recorded for 17 days. Overall, AP and IIH shapes demonstrated high levels of similarity. We identified a total of 82 stable neurons in monkey mn351, which were recorded on all 15 microwires. Despite the presence of spike activity from neurons in at least half the channels from the first day after implantation, stable recordings of the activity from individual neurons in any of the channels could be obtained no earlier than six days after implantation. Stable neuron recordings were often possible only 6–14 days after implantation. It should be noted that stable recordings of neuron activity could be obtained from all the microwires used, without exception.

Figure 3 shows a plot of the distribution of the durations of observation of the activity of individual neurons. In about half of cases, activity could be recorded from an individual

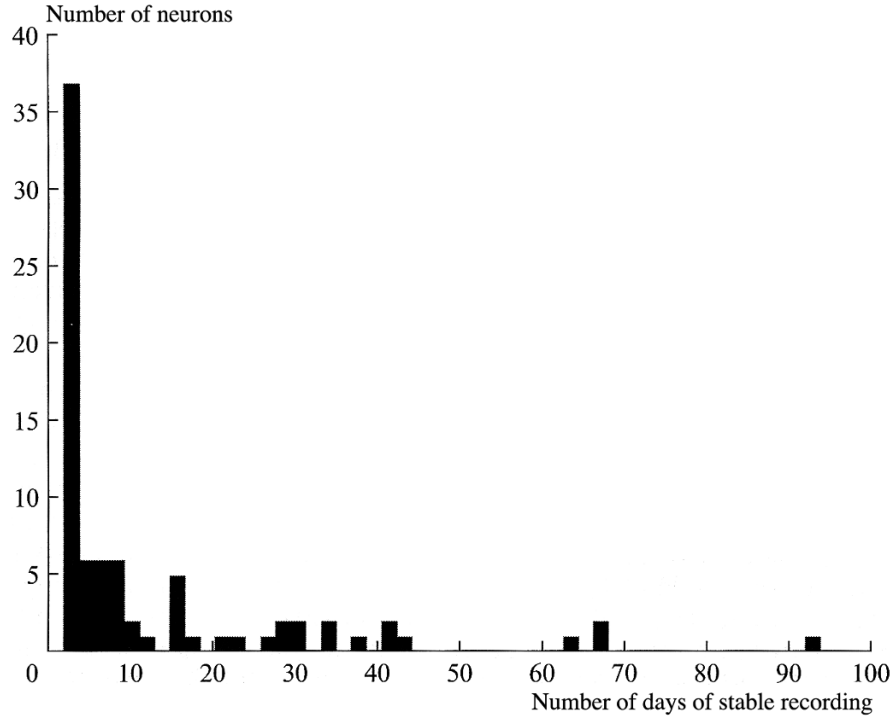

Fig. 3. Distribution of recording durations for individual neurons. Recording duration was often limited to two days, though examples of longer-duration recordings were obtained, in one case reaching 94 days.

neuron on two sequential days. In one case, stable recording was possible for 94 days. The shortness of the recording period may be due both to processes occurring in the tissues and to the characteristics of the construction and implantation of guide tubes into the skull used here. Both problems require further study and additional technical improvements.

Discussion

Reliable evaluation of the stability of activity recording from an individual neuron requires evaluation of adequate assessment criteria. In our view, conclusions regarding the stability of neuron recording must be based on parameters with the greatest possible independence from processes such as responses to changes in the stimulation situation, plasticity, and learning. There is therefore a preference for using the characteristics of spontaneous discharges. AP shape seems a reliable individual neuron feature. As shown previously, a number of groups of neurons with similar functional characteristics, including the shapes of their AP, can be observed in the motor areas [Merchant et al., 2008; Vigneswaran et al., 2011]. In addition, the use of only a small number of AP properties (for example, duration) can increase the proportion of false classifications. We therefore analyzed the similarity of AP shape with the aim of identifying the patterns required for successful classification of identical neurons.

Fraser and Schwartz [2012] suggested that stability could be evaluated in terms of four parameters: AP shape, mean discharge frequency, cross-correlations with other neurons, and IIH. In fact, most of the parameters used for assessment of recording stability provided for reliable classification of neurons, though some of these parameters could undergo changes as animals learned. An example of this is provided by changes in the tuning of monkey motor cortex neurons to movement direction during learning to control using a brain-computer interface [Taylor et al., 2002; Serruya et al., 2002]. It is not known whether other characteristics of neuron discharges change with time or in which conditions this might occur. For this reason, it follows that not all parameters available for comparison should be used, but rather that the minimum set needed for classification should be selected, i.e., the most stable of these, with classification being performed using these parameters. Our suggested approach provides a good tool for studying neuron activity recorded over prolonged periods and for studying the neurophysiological mechanisms of brain plasticity.

We observed that the possibility for stable recordings of neurons arises no earlier than six days after implantation of bundles, despite the fact that neuron spike activity was present in the channels throughout this time. these observations may point to stabilization of the environment close to the microwires. Implantation of bundles is a traumatic event for nervous tissue and triggers cellular cascades directed at restoring the blood-brain barrier to prevent possible infection and to provide trophic support to nerve cells. It is known from the literature [Polikov et al., 2005] that the acute phase of the reaction lasts 1–3 weeks after implantation, so the fact that it becomes possible to obtain recordings from individual

neurons may be evidence for completion of the acute phase of the reaction to bundle implantation. In the light of our data, investigators using chronic recording of neuron activity should plan implantation surgery in experiments in such a way that animals should not be trained to the experimental task during the two weeks following microelectrode implantation, as analysis of the stability of recording individual neurons during this period may be difficult.

It is important to discuss the limitations of our approach. It is known that the IIH parameters of neurons can change with time [Li et al., 2007]. Thus, this test does not allow a neuron to be identified on traces on different days if its IIH parameters change. This test is not suitable for cases in which IIH or AP shape undergo changes, though the method is eminently applicable to traces of spontaneous activity, as in our experiments, where there is no systematic training.

In conclusions, we will list the most significant advantages of this test for evaluating recording stability.

1. The smallest possible number of characteristics of neuron discharges is used for classification of neuron activity.

2. Reliable classification of neuron activity provides a minimum probability of errors (the probability of false negative classifications is 0.361% and the probability of false positive classifications is 3.39%).

3. The methods is universal, as its use requires only traces of spontaneous neuron activity.

4. Its use requires only a small segment of traces, of duration about 3–5 min.

5. The methods does not require complex statistical methods or high processing capacity, such that it will be possible to use the method in parallel with data recording.

6. The method is objective, i.e., any experimenter, regardless of the experiment and other factors, will come to the same conclusion using this test.

Conclusions

1. Analysis of the effectiveness of different parameters of spontaneous neuron discharges for classifying cell activity showed that use of a stability test based on only one parameter of spontaneous neuron discharges cannot be effective for classifying activity obtained a different recording periods.

2. on the other hand, use of a test taking cognizance of two spontaneous discharge parameters simultaneously significantly increases the effectiveness of the classification of recorded activity. Comparison of the shapes of interstimulus interval histograms along with comparisons based on action potential shape provided the best results.

3. Use of a test for recording stability based on comparison of action potential shape and interspike interval histogram shape allowed us to evaluate the duration of stable recording using multiple microwires: we were able to record 82 neurons whose activity was seen on traces for more than a day; the longest duration of stable recording was 94 days, with a total observation period of 115 days.

4. Our results characterize microwire bundles as the best and most reliable tool for studies involving long-term recording of activity in animals' brains. There is no doubt that the experimental approaches suggested here may be useful for developing brain-computer interfaces which in the future may be of value in treating patients with severe impairments to the motor system.

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