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Estimation of the distribution of the EMG signal amplitude

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Abstract. Quantitative analysis of electromyographic (EMG) data still demands new methods. One of the methods is based on counting occurrences of signal crossings of "noise level" (per time unit). This provides an estimator of the so called "aggregate activity." These data can be obtained by a spike trigger connected to a computer. Comparison of total activity of firing motor units in intact and affected muscles may give important information about the neuromuscular system in norm and pathology. The aggregate activity method does not seem, however, to be completely objective, because its results depend on the value of noise level selected by the experimenter. Thus, we propose a modification that allows: (1) quantitative estimation of how the results obtained with the aggregate activity method depend on the value of selected level, and (2) assessment of the distribution of the EMG signal amplitude - i.e., appearance of particular height spikes in the signal. The advantages and disadvantages of the method are discussed in the context of two experimental models.

Key words: aggregate activity, EMG, partial denervation

INTRODUCTION

Experiments dealing with the electromyographic activity (EMG) need an objective method to assess quantitatively this activity. Detection and recognition of motor unit action potential (MUAP) in EMG signal are difficult because single action potentials of different motor units are interfering. Thus, recognition of MUAPs is practically possible only at low level of activity, when a few motor units are activated. The method of quantitative evaluation of the data, based on counting number of "noise level" crossings of the signal (called an aggregate activity) was used by other authors in the case of more complex EMG activity (Fischbach and Robbinson 1969, Navarrete and Vrbová 1984, Hnik et al. 1985, Sławińska et al. 1995). Number of crossings of the noise level can be established by a spike trigger connected to a computer counting pulses. This method is not, however, completely objective since the noise is arbitrarily determined by experimenter. Thus, we propose a new method allowing the estimation of the number of crossings of different voltage levels that are set up by the computer program, and depend only on the standard deviation of the amplitude of analysed EMG signal. When necessary, the voltage levels based on other criteria can be selected by the experimenter. This method enables the assessment of the aggregate activity at different voltage levels, and using it for the comparison of the appearance of spikes with different amplitude.

To test a validity of our method we used an experimental model in which the number of motor units was surgically reduced by sectioning one of the ventral rami innervating two muscles: the extensor digitorum longus (EDL), and the soleus muscle (SOL). EDL the flexor of the hindlimb ankle is a predominantly fast muscle. It is active in locomotion during the swing phase of the step cycle. When the animal is standing, the EDL muscle is not active and the EMG signal is practically flat. SOL belongs to the group of ankle extensors. It is a tonic muscle, active both during standing, and the stance phase of the step cycle.

After partial denervation, both the number and the size of remaining motor units change. Because it is known that partial denervation induces changes in the EMG activity of EDL and SOL muscles (Sławińska et al. 1995, Tyč and Vrbová 1995) we tested the proposed method by investigation of the total EMG activity recorded from the control and partially denervated hindlimbs muscles in freely moving rats.

METHODS

Material

The effects of partial denervation on EMG activity of EDL and SOL muscles were investigated in separate experiments. The bipolar EMG electrodes were implanted into the SOL or EDL muscles of both hindlimbs in Wistar rats (for details see: Sławińska et al. 1995, Sławińska et al., in preparation). EMG electrodes were connected to the socket placed on the back of the rat (Hnik et al. 1978).

Partial denervation of EDL muscle was performed on matured rats (6 months old). A week after implantation of the electrodes the left EDL was partially denervated by section of the left L4 ventral ramus that supplies 60-80% of EDL's innervation (Albani et al. 1988). The EMG activity of EDL muscles of both hindlimbs (operated and control) was recorded and compared in freely moving rats before, and 3 weeks after the injury (for details see Sławińska et al., in preparation).

Partial denervation of the SOL muscle was achieved by section of the left L5 ventral ramus that supplies 50-70% of SOL's innervation (Fisher et al. 1989). The denervation was performed on 5 days old rats. Six months later, a week after implantation of the electrodes, the EMG activity of both (partially denervated and contralateral control) SOL muscles was recorded and compared in freely moving rats (for details see Sławińska et al. 1995).

Method of analysis

The EMG activity recorded during exploratory behaviour was chosen for the analysis (5-min peri-

ods). To compare the EMG activity of the particular affected muscle with the simultaneously recorded activity of the same muscle of contralateral unoperated leg, or to make comparison of this relation before and after injury the following procedure was used:

STEP 1

The 2-channel raw EMG activities were amplified, recorded on FM tape recorder (Racal), and digitized "off line" (12 byte A/D converter, sampling frequency 4 kHz).

STEP 2

The mean (MEAN) and standard deviation (SD) of the amplitude of the digitized EMG signal were calculated for each channel.

STEP 3

For each channel 17 levels were determined within the range of MEAN±2SD. The distance between the consecutive levels was equal to the value of ¼ SD. The SD value can be automatically calculated or replaced by an arbitrary value. In our case the MEAN and SD values were calculated for both intact EDL muscles, and the same values were used to estimate all the parameters after partial denervation. In case of early SOL denervation the automatically estimated values were used because the experimental procedure did not enable to analyse the activity of the muscle before the denervation.

STEP 4

The total number of level's crossings during whole period of analysis was counted for each level and each channel. It was assumed that the level was crossed when two consecutive samples fulfilled the following condition: that the first sample was below and the second was higher than a particular level. The opposite criterion of finding of the level crossing (i.e., that the first sample is above and the second is lower than a particular voltage level) does not influence results because the EMG signal is a continuous function of time. The Step 4 as a result, gave two tables:

$$C_i = [C_{i,1}, C_{i,2}, \ldots, C_{i,17}],\ i = 1,2 \text{ (index of channel)}$$

where $C_{i,k}$ denotes the number of crossings of level indexed "k" in the channel "i".

We chose throughout this paper index i=1 to denote the partially denervated muscle and index i=2 to denote the control muscle.

STEP 5

Two additional tables S_i (i=1,2) were defined:

$$S_{i,k} = \begin{cases} C_{i,k} & \text{for } k = 1 \text{ and } k = 17 \\ C_{i,k} - C_{i,k-1} & \text{for } k = 2, \ldots, 8 \\ C_{i,k} - C_{i,k+1} & \text{for } k = 9, \ldots, 16 \end{cases}$$

Let us assume that there is a spike in the signal starting from the MEAN value and reaching the highest voltage level (see Fig.1). The existence of such spike in a signal causes an increase of the value of all elements in C_i table over the MEAN (8-th) level: ($C_{i,9}$, $C_{i,10}$, . . . , $C_{i,17}$), but in the S_i table only the 17-th element increases. One can say that the values of the elements of the S_i (i=1,2) tables are correlated with the number of spikes of particular amplitude. Thus, the S_i (i=1,2) tables denote the distributions of the EMG signal amplitude in two compared channels. In Figure 1 an example of a short period of SOL muscle activity is demonstrated. Seventeen voltage levels are represented by horizontal lines from the lowest (1-st) through MEAN (8-th) to the highest (17-th) level. Points of crossings of the levels are marked by big black dots. On the left side of the figure are shown numbers representing appropriate values of the C_i and S_i.

STEP 6

To estimate whether the activities of two analysed muscles are similar or different, four final tables of coefficients (Γ, Ω and Γ^*, Ω^*) were introduced additionally:

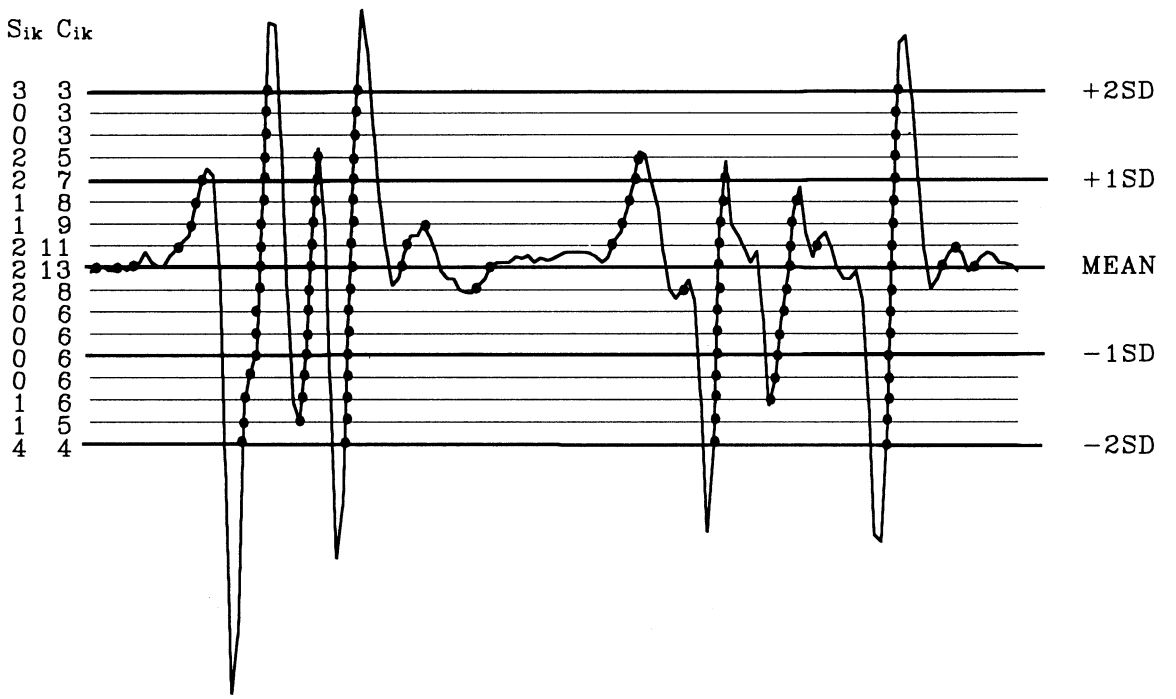

Fig. 1. Schematic drawing showing how the numbers of level's crossing are established for each level. Horizontal lines represent the levels. The points (black dots) represent the level's crossings in the analysed EMG signal found during the Step 4 of the described method (for description of the S_{ik} and C_{ik} see Methods section).

$$\Gamma_k = C_{1,k}/C_{2,k}$$

$$\Omega_k = S_{1,k}/S_{2,k}$$

where $k \in N$ and $1 \leq k \leq 17$.

However, the values of such defined coefficients before and after replacement of channels are not symmetric in respect to the value of 1 (expected for normal intact muscles). For example, if the Γ or Ω for chosen level were equal to 0.5, changing the order of the channels would gave the value of 2.0. The distance of these two values from the expected value is different (0.5 and 1.0 respectively). To make these coefficients symmetric in respect to a value of 1 the normalized ratios of Γ^* and Ω^* defined bellow were calculated:

$$\Gamma_k^* = \begin{cases} \Gamma_k \text{ for } \Gamma_k \leq 1 \\ 2 - 1/\Gamma_k \text{ for } \Gamma_k > 1 \end{cases} \qquad \Omega_k^* = \begin{cases} \Omega_k \text{ for } \Omega_k \leq 1 \\ 2 - 1/\Omega_k \text{ for } \Omega_k > 1 \end{cases}$$

After normalization, replacement of the channels does not influence the distance from the value of 1 (in our example the value of 0.5 in both cases).

RESULTS

In normal rat, analysis of EMG data recorded from EDL muscles of both hindlimbs during exploratory behaviour showed that the coefficients Γ and Γ^* were very close to the value of 1.0 in the tested range of variability (MEAN±2SD) (Fig. 2). One can say that the aggregate activity method did not show any differences between the EMG activity of examined muscles in analysed record of data in the whole tested range of variability. It means that in normal animal during spontaneous exploration both EDL muscles are active in a similar way.

The values of the coefficients Ω and Ω^* were also close to the value of 1.0, but in a range of MEAN±SD (Fig.2). In a wider range some irregular deviations can be found. The value of coefficient Ω

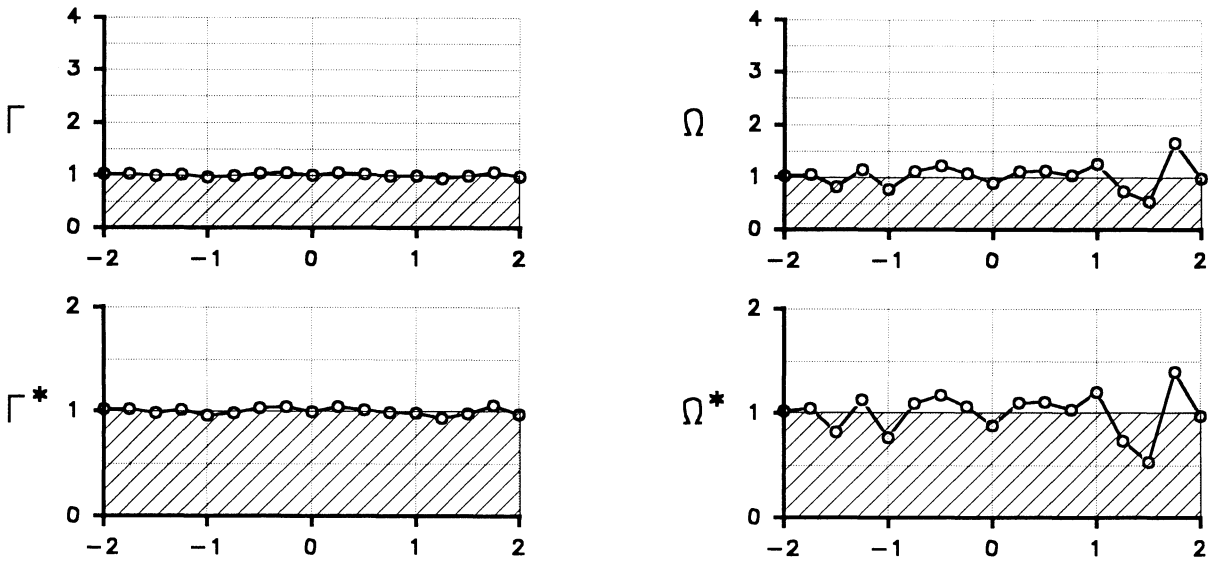

Fig. 2. Coefficients Γ, Γ^*, Ω, and Ω^* as a function of the distance from the MEAN value, calculated for the EMG activity of normal EDL muscles. Horizontal axes represent the distance from the MEAN value expressed in SD units. For example the value of 0 denotes the MEAN value (9-th level) and the value of 1 denotes MEAN+SD value (13-th level). Vertical axes represent the values of calculated coefficients Γ, Γ^*, Ω, and Ω^*. The hatched rectangle represents the area in which the values of aforementioned coefficients decrease below the value of 1, expected in this procedure for intact muscles. For full description see Methods section.

is correlated with the ratio of number of spikes of particular height in two simultaneously recorded EMG signals, while Ω^* shows the same but after normalization. Figure 2 shows that there exist slight differences in the frequency of appearance of high spikes in analysed muscles in normal rat during regular locomotion. The small difference between the EMG activities of two normal muscles is not surprising. One of the reasons of asymmetry in the activity of the analogical muscles of both hindlimbs might be different localizations of the electrodes. The other explanation might arise from the physiological differences between two hindlimb muscles, due to the right-handed dominance which was described in rats and cats (Hulborn and Malmsten 1983, Malmsten 1983). The role of the different number and size of motor units can not be excluded in explanation of aforementioned asymmetry. One should be also cautious when discussing the results obtained for high spikes because they are less frequent than the low and medium amplitude spikes. Thus, only clear and continuous changes for high spikes should be taken into account.

In rats after partial denervation of EDL muscles the coefficients Γ and Γ^* are exceeding the value of 1.0 in the whole analysed range (Fig. 3). This increase depends on the index of the level. It means that the activity of partially denervated EDL muscle (measured as the aggregate activity) was bigger than that of contralateral unoperated muscle, for all levels. From these results one can draw a conclusion that the motor units, which remained after partial denervation markedly increased the frequency of their activity (because the number of them was strongly reduced while the aggregate activity increased). Coefficients Ω and Ω^* are asymmetric with respect to the MEAN value (9-th level) in the range between ½ SD and 1½ SD (Fig. 3), but for the highest spikes we can see consistent increase. The change of polarization of either of channels does not remove this asymmetry (not illustrated) because the values in table S_1 (injured muscle) are not symmetric in respect to its MEAN while the values in table S_2 (intact muscle) are. It can be interpreted that after partial denervation the EMG activity of the injured muscle became less symmetric

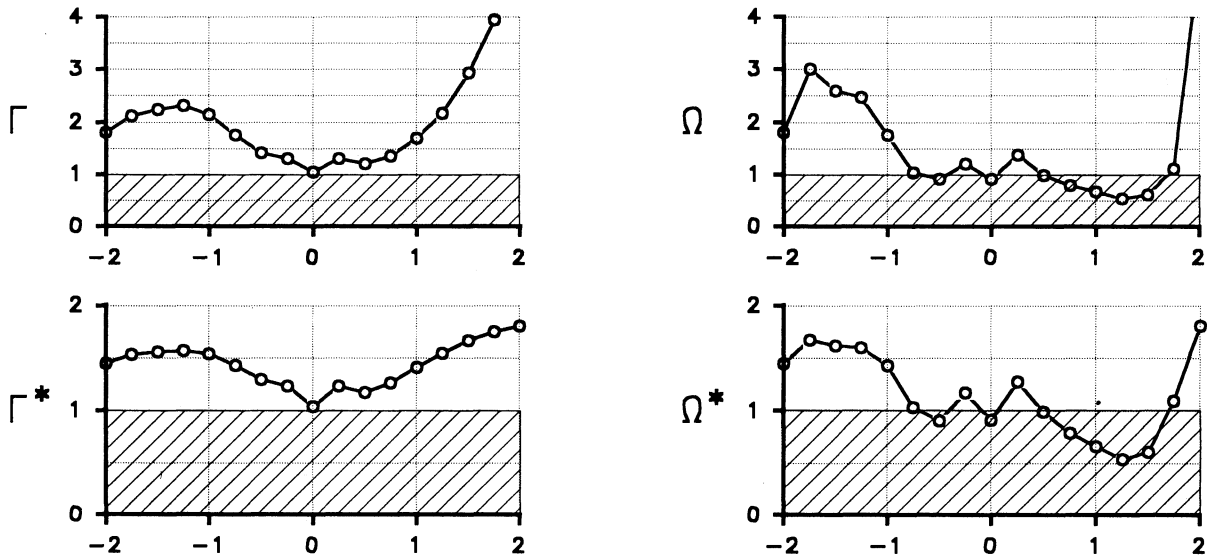

Fig. 3. Coefficients Γ, Γ^*, Ω, and Ω^* as a function of the distance from the MEAN value, calculated for the EMG activity of partially denervated and control EDL muscles (for explanation see Fig.2)

in respect to its mean, and that the number of highest spikes in its EMG signal was greater than that in the control muscle.

In rats with partial denervation of the soleus muscle changes in all four tables of coefficients Γ, Γ^*, Ω, Ω^* were consistent (Fig. 4). All coefficients were below the value of 1.0 for the signal in the range between the values of MEAN–SD and MEAN+SD. In the case of Ω and Ω^*, where smaller activity of partially denervated muscle was found, the borders were even wider. This indicated that the activity of partially denervated soleus was lower than the activity in the contralateral unoperated muscle. However, it is important to stress that the

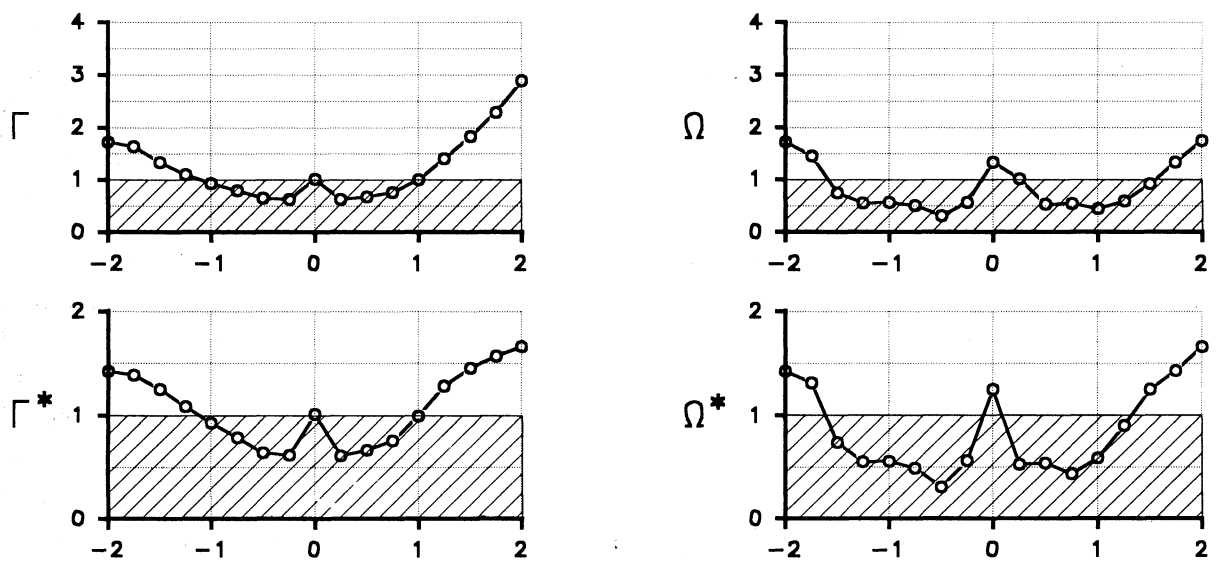

Fig. 4. Coefficients Γ, Γ^*, Ω, and Ω^* as a function of the distance from the MEAN value, calculated for the EMG activity of partially denervated and control SOL muscles (for explanation see Fig.2)

operated soleus muscles had only 30-50% of their normal complement of motor units left. The amount of activity per motor unit was higher than that in the control muscles. The same result was obtained in the past, when the aggregate activity was measured (Sławińska et al. 1995). In the present paper, however, the smaller activity of partially denervated soleus muscle was obtained only within the range MEAN±SD. Calculated in a wider range the value of Ω^* showed the increased number of highest spikes in the affected soleus than in the control muscle which is consistent with the bigger size of the remaining MUs in partially denervated muscle.

DISCUSSION

The demonstrated method allows comparison of simultaneously recorded EMG activity of two muscles. The coefficients Γ, Γ^* are the ordinary and normalized aggregate activity ratios of chosen muscles as a function of voltage level. The coefficients Ω and Ω^* correlate with the ordinary and normalized ratios of spike amplitude distributions, respectively. Although the results seem to be different in the slow and fast muscles when total aggregate activity is estimated, in both cases the partial denervation caused an increase in the activity of the remaining motor units. These data are consistent with the results previously described (Sławińska et al. 1995, 1996). Our method gave another interesting result: the EMG activity of any of partially denervated muscles (EDL or SOL) contains increased number of high amplitude MUAPs (Figs. 3 and 4), which seems to be related to the fact that the size of surviving MUs is bigger in partially denervated muscles. The mechanisms that lead to increase of the remaining MU size are different in each of our experiments. In the case of soleus the partial denervation performed on 5 days old rats caused a persistence of expanded peripheral field of the remaining MU which at the time of injury are still large (Fisher et al. 1989). In the case of adult partially denervated EDL muscle, the remaining MU increases their size by the sprouting process (Brown et al. 1981, Connold et al. 1992, Tyč and Vrbová 1995).

It is important to remember about the limitations of the presented method. The normal EMG activity is a composition of potentials of many motor units active at the same time. An additional difficulty is that the single MUAP after injury or in pathological situation can be polyphasic (Schwartz et al. 1976, Stålberg et al. 1976). Only in a case of sparse EMG activity it is possible to recognize and identify the shape of single motor unit potentials. According to the above restrictions we can only say that our procedure enables to assess the number of spikes of particular height in the EMG signal per unit of time, regardless whether they are a composition (the sum) of a few potentials or not.

Another problem which arises when we try to evaluate the EMG signal is caused by dependence of the recorded EMG activity on such experimental factors as the nature of the signal, type and the position of the electrodes, the quality of hardware, a frequency band of amplifiers or filters, etc. If the comparison of the activities is needed, it is possible to control most of these factors and make them stable from one test to another. There are, however, some differences among the electrodes, and where they are placed in the muscle. Moreover, the number of motor units in the same muscles of both hindlimbs in normal animal can be slightly different. It seems not to be the serious problem when there is the opportunity to compare the relations of the EMG activity of the same muscles of both hindlimbs before and after injury like in the case of EDL muscles in our experiment. Even if we obtained the coefficients significantly different from expected value (1.0) we would be able to show the direction and the strength of changes after partial denervation. The situation is becoming worse when the only control for injured muscle is the contralateral muscle like as in the case of SOL muscle in our situation. The only reliable solution in this situations seems to be an increase of the size of the group of tested animals.

In conclusion, the presented method of analysing 2-channel records of raw EMG activity allows to calculate tables of coefficients: Γ, Γ^*, Ω, Ω^*. Γ is the ratio between the aggregate activities of two ana-

lysed muscles within each level. Usually the triggering level is set up arbitrarily just above the noise level. The frequency of signal crossings at the particular level strictly depends on the height of this level. It means that the experimenter has to be very careful when setting up the triggering level. Our method allows to alleviate this problem, and shows how to set the levels on the basis of dynamics of analysed signal. This provides us with a possibility to analyse the signal in a wider range of amplitudes.

The other advantage of the described method is that the values of the Ω coefficients correlate with the ratio of number of spikes of particular height in two analysed channels. The value of ratios shows, whether the number of determined level crossings for one signal is lower, higher or the same as for the second one. They may also show whether the compared signals are symmetric in respect to their means. The described above coefficients can help to assess the activity of high, medium or low spikes in the EMG activity.

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