Electrophysiological and anatomical estimation of the number of motor units in the monkey extensor digitorum brevis muscle

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SUMMARY The electrophysiological technique used to estimate the number of motor units in man (McComas et al., 1971b) was tested in six monkeys. The number of motor units was estimated electrophysiologically in the extensor digitorum brevis (EDB) after deafferentation by excision of the lumbo-sacral dorsal root ganglia. In five animals, the mean number of motor units obtained by the technique was in good agreement with the number of alpha motor fibres counted in the nerve to EDB. However, the results obtained in one animal with partial denervation of the EDB indicate that the technique might be unreliable, at least at certain stages, after a peripheral nerve lesion.

McComas et al. (1971b) described an electrophysiological technique to estimate the number of motor units in the extensor digitorum brevis muscle (EDB) of man. They observed that small graded changes of the electrical stimulus to the deep peroneal nerve at the ankle induced an incremental response in the evoked action potential of the EDB. Assuming that each increment of voltage was due to the excitation of one motor unit, they divided the average amplitude of the increments into the M response evoked by a maximal nerve stimulation to obtain the approximate number of motor units within the muscle. Findings by this technique indicated a loss of functioning motor units in patients suffering from various types of muscular dystrophies (McComas et al., 1971a; McComas et al., 1971c; Sica and McComas, 1971), myasthenia gravis (McComas et al., 1973a), as well as in hemiplegic (McComas et al., 1973b) and elderly subjects (Campbell et al., 1973).

However the validity of the technique has been questioned (Feasby and Brown, 1974; Brown and Milner-Brown, 1976), and doubts have been expressed regarding the significance of the results obtained in patients (Engel and Warmolts, 1973; Panayiotopoulos et al., 1974).

In order to test the accuracy of the motor unit counting technique, attempts have been made in man (McComas et al., 1971b) and animal (Eisen et al., 1974) to compare electrophysiological evaluations of the number of motor units in a muscle to histological counts of alpha fibres in the motor nerve. However, such an approach is limited by the difficulty of estimating the proportion of sensory and motor fibres in non-deafferented nerves. For this reason, we have used the technique of McComas in the present study to determine the number of motor units in both the normal and deafferented EDBs of monkeys, and we have compared these estimates with the histological counts of alpha motor fibres in the deep peroneal nerve, which supplies this muscle.

Methods

The experimental animals were Macaca mulatta. In these monkeys as in man the EDB is supplied by the deep peroneal nerve which divides at the ankle into two branches: the purely sensory medial branch which innervates the skin of the first interdigital cleft, and the lateral motor branch, which supplies the EDB and occasionally sends a terminal twig to the dorsal interosseous muscles (Fig. 1). Preliminary experiments showed that only L6 and L7 motor roots contributed to the innervation of the EDB (Fig. 2).
Electrophysiological and anatomical estimation of motor units

ELECTROPHYSIOLOGICAL TECHNIQUE

Before each recording session, the animals were anaesthetised with sodium pentobarbitone injected intraperitoneally. The protective boot was taken off and the skin shaved and cleaned. The stimulating electrodes consisted of a pair of silver screws padded with felt soaked in a sodium chloride solution (0.9%). They were inserted in a plastic holder 12 mm apart and positioned on the skin at the ankle over the deep peroneal nerve, the cathode being distal to the anode. A stimulator (DISA Multistim) was used to deliver rectangular pulses of variable voltage and 50 μs in duration at a frequency of 0.5 Hz.

The surface recording electrodes were made of thin silver strips 25 mm long and 5 mm broad, coated with conductive jelly. The active electrode was placed on the endplate zone of the muscle where the amplitude of the evoked response was maximal and its latency shortest. The reference electrode was attached to the sole of the foot and an earth (ground) electrode was placed around the leg.

The muscle responses were amplified, recorded on a storage oscilloscope, and photographed. The contours of the potentials were carefully drawn on transparent graph paper. In monkeys, as in man, a gradual increase in the strength of stimulation to the deep peroneal nerve at the ankle evokes a gradual increase in the response recorded in the EDB. Near threshold the increases in response are 'all or nothing' increments presumed to arise from the recruitment of individual alpha motor axons. However, above the level of the 'maximum incremental response' these steps are not distinguishable. Figure 3 is an example of recordings made during one session. In order to calculate the mean motor unit voltage, the peak to peak amplitude of the largest incremental response (in this case 954 μV) was divided by the number of increments (10). The number of motor units (88) was obtained by dividing the mean motor unit amplitude (95.4 μV) into the amplitude of the muscle response evoked by supramaximal stimulation (8.4 mV). In five animals (PA, PB, PD, PE, PF) these measurements were repeated three to 23 times during each

SURGICAL TECHNIQUE

Six adult animals (PA, PB, PD, PE, PF, and PG) of both sexes were anaesthetised with an intravenous injection of sodium pentobarbitone. Under sterile conditions a lumbo-sacral laminectomy was performed on the right side. The dura mater was opened and the L5, L6, L7, and S1 dorsal roots were sectioned. Each dorsal root ganglion was isolated from the ventral root fibres and removed. Before returning the animals to their cages, the deafferented leg was protected by a boot made of gauze and rubber bands extending up to mid-calf.

Fig. 1 Diagrammatic representation of terminal distribution of deep peroneal nerve in the monkey. EDB, extensor digitorum brevis; D1, dorsal interosseous.

Fig. 2 Potentials evoked in EDB after supramaximal ventral root stimulation. Only L6 and L7 contributed to motor innervation of the muscle. Negativity is upward.

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branch of the superficial peroneal nerve. The roots and spinal nerves were also dissected to be examined for residual dorsal root ganglion cells. The nerves were cut in pieces approximately 1 mm in length, post-fixed in 2% osmic acid, and prepared according to standard techniques for inclusion in Epon. Thin sections were stained with paraphenylenediamine, examined by phase microscopy and photographed. The fibre size histogram of the motor branch of the deep peroneal nerve was obtained from enlarged photomicrographs ($\times1000$) using a particle size analyser (Zeiss TGZ3). Seven different levels were studied on the right deafferented side, from the ankle to the muscle in order to evaluate the incidence of axonal branching. On the left normal side, fibre size histograms were constructed at three different levels.

**Results**

**RIGHT DEAFFERENTED EDB**

*Electrophysiological estimates of the number of motor units*

The results from all recording sessions were pooled for each animal and the average number of motor units ($\pm$ one standard deviation) was found to be respectively: $97 \pm 20$ (PA), $92 \pm 17$ (PB), $86 \pm 17$ (PD), $99 \pm 15$ (PE), $65 \pm 13$ (PF).

The values obtained on different recording sessions for each of the five animals are presented in Table 1. The variations which are seen on successive sessions show no obvious systematic trend. The discrepancy between the largest and the smallest estimates ranged from 19% (PE) to 43% (PF) with an average of 32%. In the animal with severe L7 ventral root lesion (PG) there was a marked difference between the first and the last session. The estimated number of motor units was initially low ($35 \pm 2$) and increased progressively to a final value of $102 \pm 19$ twelve weeks after the operation (Fig. 4).

**Table 1 Mean number of motor units in EDB right side—after deafferentation**

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>$72 \pm 16$</td>
<td>$117 \pm 7$</td>
<td>$85 \pm 6$</td>
<td>$99 \pm 8$</td>
<td>$92 \pm 25$</td>
<td>$97 \pm 20$</td>
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<td>N: 5</td>
<td>N: 10</td>
<td>N: 10</td>
<td>N: 11</td>
<td>N: 3</td>
<td>N: 39</td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>$90 \pm 8$</td>
<td>$95 \pm 19$</td>
<td>$81 \pm 7$</td>
<td>$124 \pm 8$</td>
<td>$92 \pm 17$</td>
<td>$92 \pm 17$</td>
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<td>N: 7</td>
<td>N: 7</td>
<td>N: 12</td>
<td>N: 4</td>
<td>N: 30</td>
<td>N: 30</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>$79 \pm 13$</td>
<td>$85 \pm 12$</td>
<td>$81 \pm 9$</td>
<td>$91 \pm 11$</td>
<td>$86 \pm 17$</td>
<td>$82 \pm 17$</td>
</tr>
<tr>
<td>PE</td>
<td>$92 \pm 13$</td>
<td>$100 \pm 16$</td>
<td>$114 \pm 8$</td>
<td>$94 \pm 11$</td>
<td>$99 \pm 15$</td>
<td>$99 \pm 15$</td>
</tr>
<tr>
<td>PF</td>
<td>$86 \pm 5$</td>
<td>$68 \pm 11$</td>
<td>$49 \pm 3$</td>
<td>$54 \pm 5$</td>
<td>$63 \pm 6$</td>
<td>$65 \pm 13$</td>
</tr>
</tbody>
</table>
Electrophysiological and anatomical estimation of motor units

Small myelinated fibres (2–3 μm in diameter) were observed. It is unlikely that they represent regenerating fibres, because such fibres could not have reached the ankle even after a period of 12 weeks if one accepts the growth rate of axons to be 2.5 to 3.5 mm/day (Gutmann et al., 1942), since the distance between the operated spinal roots contributing to the nerve and the ankle is about 350 mm. Furthermore, they were observed in one animal killed only five weeks after the operation. They may, therefore, represent postganglionic sympathetic myelinated fibres or else residual afferent fibres with cell bodies located very distally in the proximal part of peripheral nerves (Coggeshall et al., 1974) since no ganglionic cells were found in serial sections of roots and spinal nerves on the operated sites. The ventral root fibres seemed well preserved with the exception of the animal PG in whom one root (L7) supplying the EDB had been severely injured during the surgical deafferentation and showed no signs of regeneration (Fig. 6).

Histological counts of the number of alpha motor fibres
Histological examination confirmed that, after deafferentation, there were no residual large afferent myelinated fibres left in the sensory nerves examined. In some of them, such as the sensory branch of the deep peroneal nerve (Fig. 5), a few

Fig. 4 Estimated numbers of motor units in EDB muscle of monkey PG at different times after deafferentation.

Fig. 5 Light micrographs of sections from deep peroneal nerve in one of the deafferented animals (PE). There are no large myelinated fibres remaining in the sensory branch in contrast to the motor branch.
In all cases, the fibre size histograms for myelinated fibres in the motor branch to the EDB were clearly bimodal, indicating that two populations, one made of large alpha fibres and the other of small gamma fibres (Fig. 7) were present. In two animals (PB and PF) terminal branches innervating the interossei and containing respectively 37 and 40 large myelinated fibres were subtracted from the counts obtained at the ankle. The number of myelinated fibres innervating the EDB was as follows: 130 (PA), 107 (PB), 94 (PD), 99 (PE), 76 (PF), 48 (PG).

These values were fairly stable from one level to the next (Fig. 7), and observation was made that the alpha fibres divided only a few millimetres (usually 5) from the motor point.

Comparison of results
Figure 8 compares, for each animal, the number of alpha motor fibres, the mean electrophysiological motor unit estimate obtained during each recording session (black columns), and the average of all these estimates ± one standard deviation (white columns). In four animals (PB, PD, PE, PF), the histological count of alpha motor fibres fell within the range of the average electrophysiological estimates, the mean difference between the two sets of values being only 9%. For animal PA, there was a larger discrepancy between the two values which could be due to an overestimation of the number of alpha motor fibres innervating the EDB, since no search was made for a branch ending in the interosseous muscles. In the last animal (PG) with L7 ventral root injury, there were only 48 alpha motor axons innervating the EDB, which is about half the number found in the others. This was reflected by the electrophysiological motor unit estimates obtained throughout five recording sessions carried out over a postoperative period of 70 days (Fig. 4). However, during the following 20 days four sessions gave much higher values which were about twice the anatomical count.

LEFT (NORMAL) EDB
Electrophysiological estimates of the number of motor units
An estimate of the number of motor units was obtained in the left EDB (normal side) in five animals. The average number of motor units (±
Electrophysiological and anatomical estimation of motor units

![Graph](image)

**Fig. 8** Estimated numbers of motor units in EDB muscles of each of the six deafferented animals. Black columns: mean estimate obtained during each recording session; white columns: mean number for all sessions ± one standard deviation (bars). Horizontal hatched lines indicate number of alpha motor fibres counted in the nerves.

one standard deviation), calculated from 20 determinations, was found to be respectively: 121±24 (PA), 112±15 (PB), 136±27 (PD), 130±18 (PE), 100±9 (PF). These values were higher than on the right deafferented side (average difference of 27%), and the amplitude of the supramaximal responses was also greater with an average difference of 36% between the two sides.

**Histological counts of the number of alpha motor fibres**

Fibre size histograms obtained from the motor branch to the EDB were bimodal with a group of large and a group of small myelinated fibres. Subtraction was made in two animals (PB and PF) of the fibres innervating the interosseous muscles, and the number of large myelinated fibres was divided by two (Cooper 1966) in order to obtain the approximate number of alpha motor fibres. The values were as follows: 168 (PA), 135 (PB), 152 (PD), 145 (PE), 165 (PF).

**Comparison of results**

In all animals, the electrophysiological motor unit estimates were lower than the anatomical counts (Table 2). In three of them (PB, PD, PE) the difference was minimal (average 12%). It was more pronounced in two animals (PA, PF) in whom one cannot rule out a genuine overestimation of the number of alpha motor fibres innervating the EDB. In animal PA no search was made for a terminal branch ending in the interosseous muscles whereas in animal PF this branch divided profusely but only one fascicle could be dissected.

**Table 2** Left EDB muscle

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean number of motor units</th>
<th>Number of alpha fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>121±24</td>
<td>168</td>
</tr>
<tr>
<td>PB</td>
<td>112±15</td>
<td>135</td>
</tr>
<tr>
<td>PD</td>
<td>136±27</td>
<td>152</td>
</tr>
<tr>
<td>PE</td>
<td>130±18</td>
<td>145</td>
</tr>
<tr>
<td>PF</td>
<td>100±9</td>
<td>165</td>
</tr>
</tbody>
</table>

**Discussion**

In spite of the ready accessibility of peripheral nerves and muscles to histological and electrophysiological examination, neuromuscular disorders are often difficult to diagnose. Nerve biopsies are commonly restricted to a few cutaneous nerves and can be done only once. Information obtained from muscle biopsies may be insufficient for diagnostic purposes. Routine electromyography has also several limitations. For example, there are instances when it cannot help to decide whether a disease is myogenic or neurogenic in origin since fractionation of motor units may occur in both conditions, giving rise to the same pattern of short brief action potentials (Engel, 1975). Above all, none of the conventional electrophysiological techniques quantifies the number of functioning motor units in a muscle.

Therefore, the motor unit counting technique described by McComas was considered to be a
Major tool in clinical electromyography. However, the reliability of this method is questionable, considering the wide variability of the motor unit counts reported in normal subjects (McComas et al., 1971b). Several potential sources of error were identified by the authors as explaining this scattering of values. One of them recently emphasised by Brown and Milner-Brown (1976) was the likely existence of axons with the same threshold for electrical activation leading to alternate firing of motor units instead of the expected orderly recruitment. They also considered the possibility that the 10 or 11 motor units used to calculate the mean potential amplitude represented an inadequate sampling of the population of motor units. With relevance to this hypothesis, motor units whose action potential voltage was many times larger than any of those activated near motor threshold have been found in the thenar, first dorsal interosseous, and EDB muscles (Feasby and Brown, 1974; Brown and Milner-Brown, 1976). In patients with muscular dystrophies, Panayiotopoulos et al. (1974) pointed out another deficiency of the technique: its inability to discriminate small amplitude 'myopathic' potentials close to the instrumental noise level. Ballantyne and Hansen (1974) proposed a computerised analysis of the incremental responses for a better recognition of motor units but this approach is still beyond the reach of most laboratories.

Nevertheless, the need to improve the originally described motor unit counting technique depends very much on the degree of accuracy of the results it produces. This aspect has so far been poorly documented. For this reason, in the present study we have compared the electrophysiological estimates of the number of motor units in the deafferented and normal EDBs of monkeys with the histological count of alpha motor fibres supplying this muscle, assuming that one motor unit is innervated by one motor axon (Brown and Matthews, 1960; Buchthal, 1960; McPhedran et al., 1965).

After dorsal root ganglionectomy, histological studies confirmed that there were no residual large afferent nerve fibres in the distal portion of the lower limb. In all cases, the curve of distribution of diameter for myelinated fibres in the motor branch to the EDB was clearly bimodal, with a sharp demarcation between small and large myelinated fibres, the latter being considered as alpha motor axons. Needless to say, these values do not represent the exact number of motor axons existing in an intact nerve since the surgical dorsal root ganglionectomy implies partial damage to the motor root fibres (Boyd and Davey, 1966; Gilliatt, 1966; McLeod and Wray, 1967; Wray, 1969). Although there was histological evidence of severe injury to a ventral root supplying the EDB in only one animal (PG), for the others partial damage to the motor innervation of the EDB may explain why on the operated side we found on average a 36% reduction in the amplitude of the supramaximal responses and a 27% diminution in the electrophysiological estimates compared to the normal side.

Axonal branching is another factor worth considering since it is a potential source of error when using anatomical criteria to determine the number of motor units in a given muscle. Eccles and Sherrington (1930) and Wray (1969) have documented that it increases with proximity to the muscle. In our study serial counts of large myelinated fibres in the deep peroneal nerve at the ankle remained fairly stable over distances up to 30 millimetres. Division was observed only at a few millimetres (approximately 5) from entry into the muscle. This, of course, does not exclude the possibility of a division at a site proximal to the ankle. If so, when reaching this level, the divided fibres would fall into the group of either large or small myelinated fibres. In the first case, at the level of the ankle where the stimulation was applied, they would probably behave electrophysiologically as independent axons even though innervating only part of a motor unit. In the second case one would expect the anatomical counts to be much lower than the electrophysiological results. This was not the case. In four animals (PB, PD, PE, PF) the electrophysiological estimates of the number of motor units tended to be lower than the anatomical counts. However, the discrepancy between the two sets of values was relatively small, the overall mean electrophysiological estimate being only 9% inferior to the anatomical count. In the first animal (PA), there was a large difference which could be due to an overestimation of the number of motor axons supplying the EDB, since in that particular animal no search was made for a terminal branch ending in the interosseous muscles.

The last animal (PG) presents a special interest since it is the only one in which histological evidence was found of a severe injury to a ventral root (L7) innervating the EDB. In this animal a satisfactory correlation was observed between anatomical and electrophysiological data during the first 70 days after the operation. During the last 20 days, there was a marked increase of the electrophysiological estimates reaching values of about twice the anatomical count of motor axons.
To explain this finding it is proposed that during this second period a reorganisation of motor unit was taking place through collateral sprouting. In such a situation, precarious transmission in certain newly-formed neuromuscular junctions (Stålberg and Ekstedt, 1973) could produce motor unit potentials of variable voltage and which could change from one stimulation to the next giving rise to 'false' increments. If so, the technique of McComas, which relies on the stability of the incremental responses, could be inapplicable at certain stages in case of partial denervation.

On the non-deafferented side, the approximate number of alpha motor axons supplying the EDB was obtained by dividing by two the population of large myelinated fibres in the motor branch of the deep peroneal nerve at the ankle. With this criterion mentioned by Cooper (1966) a good correlation between electrophysiological and anatomical counts of motor units has been found in the EDB muscle of man (McComas et al., 1971b), and in the soleus of rat (Eisen et al., 1974). In our study, in three animals (PB, PD, PE) there was a close anatomophysiological correlation, the electrophysiological estimates being on average only 12% inferior to the anatomical determinations. The other two animals (PA and PF) showed larger differences with estimates respectively 39% and 28% inferior to the anatomical counts. As mentioned earlier there is reason to believe that, in these animals, the anatomical count was overestimated and included motor axons innervating the interosseous muscles.

In conclusion, in normal and deafferented EDBs of monkeys a satisfactory correlation was observed between anatomical and electrophysiological estimates of the number of motor units using the technique of McComas. However, the discrepancy between the values noted in one animal with a severe lesion of the motor innervation of the EDB raises doubts about the reliability of the technique in some cases of partial denervation.

References


McComas, A. J., Sica, R. E. P., and Campbell, M. J. (1973a). Numbers and sizes of human motor units...


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