

Surface area of motor end plates in fast and slow twitch muscles of the rabbit

P. L. R. DIAS

*University Department of Neurology, Institute of Neurological Sciences,
Southern General Hospital, Glasgow*

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INTRODUCTION

In most animals individual muscles are composed of a mixture of red and white muscle fibres, but in the rabbit some muscles, like the soleus, are pure red (Kruger, 1952; Henneman & Olson, 1965) while others, like the gastrocnemius, are regarded as pure pale muscle (Roberts, 1916). Histochemical examination has shown that the soleus muscle consists almost entirely of type I fibres (Dubowitz, 1968) having a high concentration of oxidative enzymes essential for aerobic metabolism. In the 'fast' muscles the type II fibres which have high glycogen levels and high phosphorylase activity predominate.

Coërs (1955) reported that rabbit 'slow' muscles like the soleus contained motor end plates with subneural apparatuses of a mean diameter significantly greater than those in the tibialis anterior and gastrocnemius, which are chiefly 'fast' muscles. His conclusions, however, were based on a study of only about 50 end plates, and so it was decided to study a much larger sample and to measure the surface area of contact of the terminal arborization rather than the overall diameter.

Denny-Brown (1929) and Creed, Denny-Brown, Eccles, Liddell & Sherrington (1932) showed that the chief extensor muscle groups of the hind limbs of all mammals could be divided into a rapidly contracting superficial component and a slowly contracting deep component. Gordon & Philips (1949) demonstrated that the superficial motor units of the cat's tibialis anterior had a shorter contraction time than the deeper units, and they showed that the superficial pale component could be easily distinguished from the deep red component of the muscle. A similar arrangement was looked for in the rabbit, and the sizes of the end plates in these two components of the muscle were investigated.

Denny-Brown (1929) demonstrated that though the gastrocnemius is a quick-contracting pale muscle in the cat and rabbit, the medial head of the gastrocnemius had a longer contraction time than the lateral head, so possible differences in the sizes of the end plates in the fast and slow heads of this muscle were also sought.

The rabbit was chosen for these studies because of the marked differentiation of its limb muscles into almost pure red and pure pale muscles. The soleus and flexor digitorum longus (FDL) are respectively the slowest and fastest contracting calf muscles of the cat (Buller & Lewis, 1965). In the rabbit, however, the bellies of the flexor hallucis longus (FHL) and FDL are fused and the whole muscle is referred to

as the FDL (Young, 1957; Dubowitz, 1967). Hence the soleus was chosen as the representative 'slow' muscle and the FDL as the representative 'fast' muscle when comparing motor end plate sizes.

Of the various methods available for demonstrating motor end plates, intravital staining with methylene blue is regarded as providing the most accurate picture of the conditions in intact living tissue because artefacts and shrinkage effects are minimal (Weddell & Glees, 1941). This method was therefore employed in the present study for the visualization of motor end plates.

METHODS

Twenty two normal adult male rabbits (New Zealand White), weighing between 2.5 and 3.85 kg, were anaesthetized with pentobarbitone sodium (Nembutal). The initial dose of 40 mg/kg was injected into an ear vein, while subsequent doses were given intraperitoneally.

The skin and deep fascia over the muscles to be examined were incised and the terminal innervation zone was located by stimulating a superficial fasciculus of the muscle with a fine pointed electrode (cathode) using an electrical square-wave pulse of 1–10 volts and 0.1 msec duration, at a rate of one per second. The anode was placed on the tendon. The strength of the stimulus was gradually increased until a contraction of the fasciculus was observed. The point where the maximum visible contraction is seen under the cathode is the end plate zone. The muscles of both hind limbs were studied and the motor end plates were demonstrated by intravital staining with methylene blue.

Some 20–30 ml of a 0.015 % solution of freshly prepared vital methylene blue in normal saline were gently injected into the end plate zone of the muscle using a 2 in. long, 26 gauge needle. After the injection, five minutes were allowed for staining to take place and then narrow strips of muscle were gently teased out. The strips were placed on gauze moistened with saline and exposed to the air for one hour, during which time the strips were repeatedly sprinkled with normal saline and turned over. They were placed overnight at room temperature in a filtered saturated aqueous solution of ammonium molybdate. They were then thoroughly washed in three changes of distilled water, placed between two glass slides and pressed firmly by means of a rod covered with a piece of rubber tubing, as described by Wilkinson (1929).

The flattened muscle strips were placed in methylated spirits for two minutes, then in absolute alcohol for three minutes and again for another three minutes in absolute alcohol. Specimens were cleared in xylol and mounted in 'Harleco' synthetic resin. The mounted preparations were kept in the dark and photographed within one week using 35 mm film in an 'Orthomat' camera. Every end plate which was seen clearly in frontal view and which was well separated from its neighbours was photographed. Selection was exercised only when the end plate appeared inadequately stained or when the whole end plate could not be clearly focused. The negative was enlarged to give a final magnification on the print of $\times 400$.

The area occupied by the terminal arborization was outlined as described by Coërs (1955) and the surface area of the outlined end plate was measured. The measuring device was a transparent ester grid upon which was inscribed a rectangle

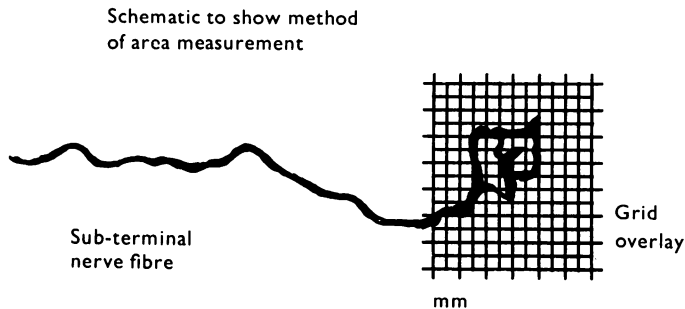


Fig. 1. Schematic drawing showing the measurement of the area of the motor end plate.

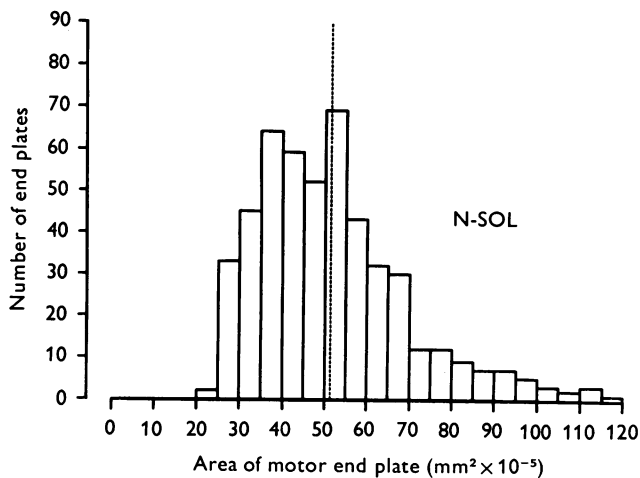


Fig. 2. Histogram of the area of motor end plates in the normal soleus muscle. (Dotted vertical line indicates mean.)

40 mm by 50 mm which was further divided into mm squares. This measuring grid, which was more than adequate to measure even the largest end plate, was placed over the photograph containing the outlined end plate and the total number of squares within the enclosed area was counted (Fig. 1).

The outlined surface area of the motor end plate, though giving an approximate figure of the synaptic surface, represents the size of the myoneural junction, which is the area of contact between the termination of the motoneuron and the muscle membrane and is a far better index than the measurement of diameter. The method used does not eliminate all artefacts of staining or shrinkage and does not represent a total measurement of functional synaptic area but is considered accurate enough for a comparative study such as this.

The motor end plates in the hind limb muscles of 22 normal rabbits were examined. The muscles studied were: soleus (SOL), flexor digitorum longus (FDL), medial head of gastrocnemius (MG), lateral head of gastrocnemius (LG), superficial component of the tibialis anterior (ATS) and deep component of the tibialis anterior (ATD). The ATS muscle fibres were those removed from the subcutaneous surface of the tibialis

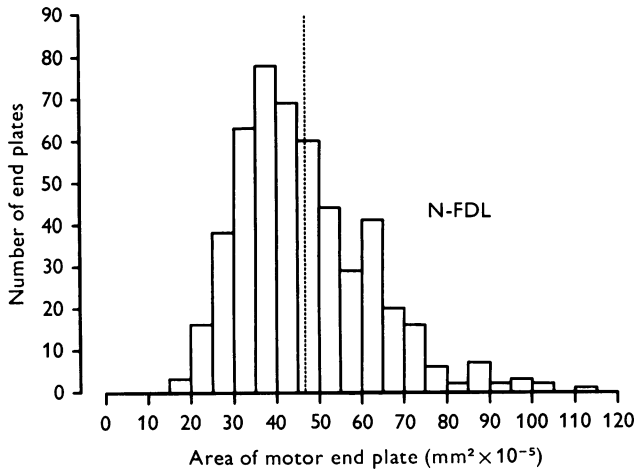


Fig. 3. Histogram of the area of motor end plates in the normal FDL. (Dotted vertical line indicates mean.)

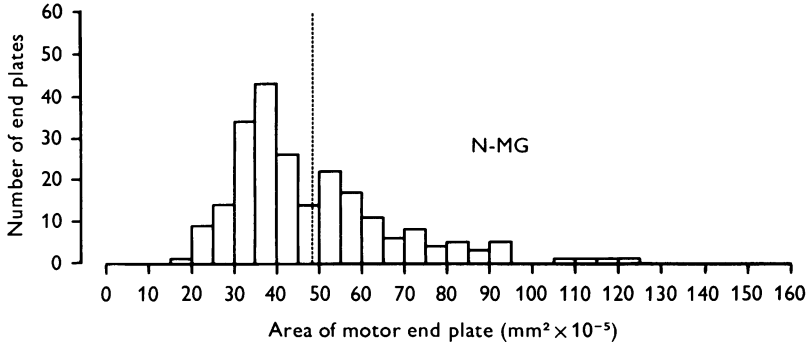


Fig. 4. Histogram of the area of motor end plates in the medial head of the normal gastrocnemius muscle. (Dotted vertical line indicates mean.)

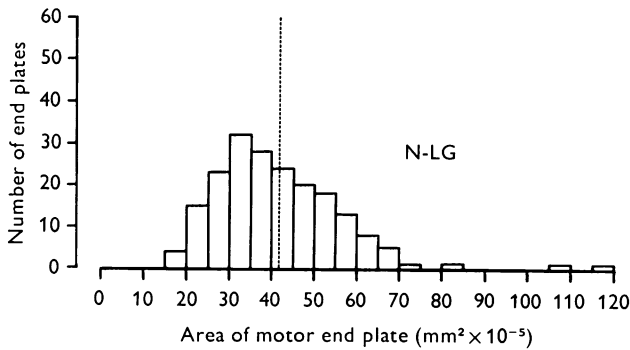


Fig. 5. Histogram of the area of motor end plates in the lateral head of the normal gastrocnemius muscle. (Dotted vertical line indicates mean.)

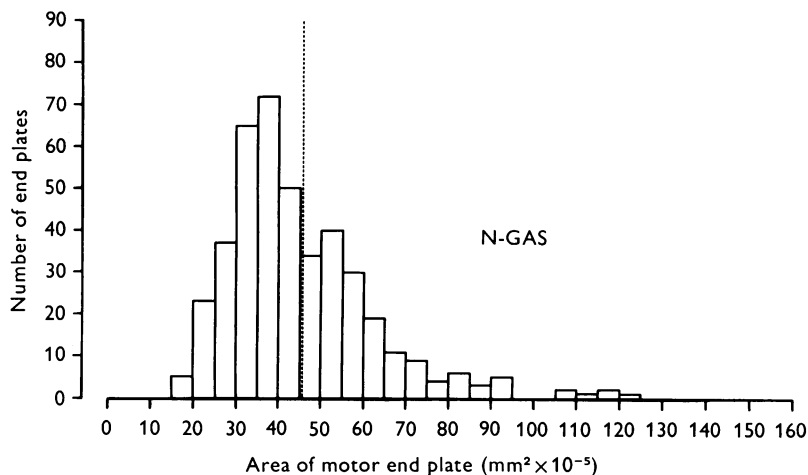


Fig. 6. Histogram of the area of motor end plates in the normal gastrocnemius muscle. (Dotted vertical line indicates mean.)

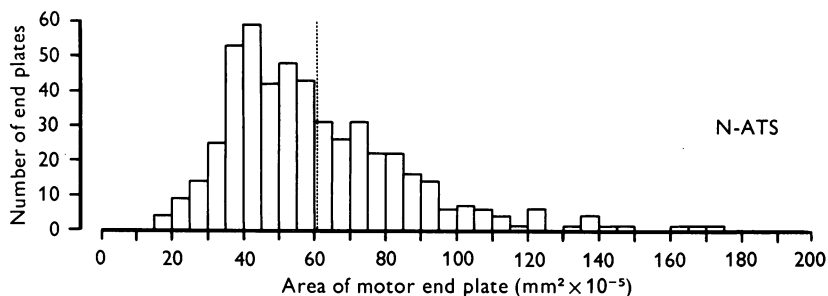


Fig. 7. Histogram of the area of motor end plates in the superficial component of the normal tibialis anterior muscle. (Dotted vertical line indicates mean.)

anterior, while the ATD were muscle fibres removed from the deep surface of that muscle.

All animals studied were 15 weeks old because it was considered desirable that all should be at the same stage of maturation. This necessarily involved a wide range of body weights, but this was considered acceptable as the research involved comparison between two types of muscle and therefore absolute end plate areas were less important than relative areas.

RESULTS

The surface areas of 500 motor end plates were measured in FDL, ATS, ATD and 496 from SOL, but a smaller sample of end plates were measured in MG and LG. The data are presented in the form of frequency block diagrams (Figs. 2–8) in which the number of end plates in each size group is plotted against the surface area of the motor end plate. The mean surface area of the motor end plates of these muscles is shown in Table 1.

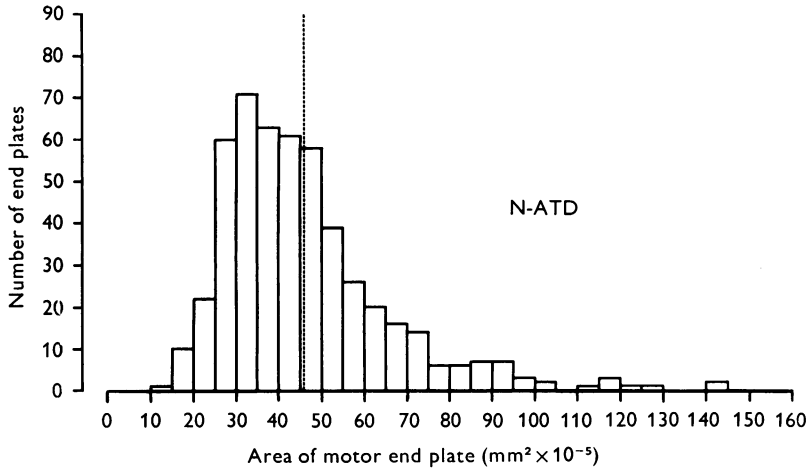


Fig. 8. Histogram of the area of motor end plates in the deep component of the normal tibialis anterior muscle. (Dotted vertical line indicates mean.)

Table 1. Mean surface area of normal motor end plates ($\text{mm}^2 \times 10^{-5}$)

Muscle	<i>n</i>	<i>N</i>	<i>A</i>	s.d.	Range	<i>P</i> (<i>t</i> -test)
N-SOL	9	496	51.1	18.3	25.0–118.8	0.0001
N-FDL	9	500	46.9	15.6	20.0–111.3	
N-MG	4	225	48.3	18.7	18.8–121.3	0.0001
N-LG	4	194	41.8	14.5	18.8–116.3	
N-GAS	4	419	45.2	17.5	18.8–121.3	
N-ATS	11	500	60.4	25.6	18.8–185.6	0.00001
N-ATD	16	500	46.4	19.7	13.1–143.8	

The widespread variations in the morphology of normal motor end plates are reflected in the broad scatter of the surface areas of these end plates. It must be emphasized that the standard deviation was high for all the muscles examined, indicating a large spectrum of end plate sizes. Although the soleus muscle is believed to be a homogeneous red muscle (Kruger, 1952; Henneman & Olson, 1965), having fibres which are relatively uniform in diameter and differing from one another much less than the fibres of the tibialis anterior and gastrocnemius, nevertheless the areas of the motor end plates in the soleus muscle showed as big a variation as those of other muscles. This wide distribution in the sizes of the end plates could be due to the varying stages of maturation of the end plate, for as Barker & Ip (1966) pointed out, motor end plates do not maintain a fixed morphology because of their periodic replacement. The small end plates studied in the present series could well be young and sprouting. Carey (1944) explained the high coefficient of variation in the size of normal motor end plates on the basis of 'functional amoeboidism'. But, in spite of these factors affecting the size of normal motor end plates, the motor end plates of the slow muscle fibres have a mean surface area which is significantly larger than those of the pale fibres.

The outstanding feature of the normal motor end plate was the great variability of the terminal filament network. Even in a particular muscle of a particular animal no end plate was ever exactly like another. On account of this marked pleomorphism it is not feasible to distinguish motor end plates of fast and slow muscles on morphological grounds.

DISCUSSION

The study of SOL and FDL motor end plates confirms the findings of Coërs (1955) that, in the rabbit, the slow soleus muscle has motor end plates whose mean surface area is significantly greater than those in the fast FDL.

The mean surface area of the motor end plates in the gastrocnemius was significantly smaller than the mean surface area of the soleus end plates, and the motor end plates in the faster LG were significantly smaller than those in the slower MG.

Hence it appears that in the muscle examined there is some relationship between the speed of contraction of the muscle and the mean surface area of the motor end plate, for slow muscles contain motor end plates whose mean surface area is significantly greater than those of the fast muscles. The only exception was found in the tibialis anterior muscle.

The tibialis anterior muscle of the rabbit, as in the cat, has well-defined superficial pale and deep red components easily separable and distinguishable from each other. The motor end plates in the superficial pale component of this muscle had a mean surface area significantly greater than the mean surface area of the motor end plates in the deep red component. The end plates in the ATS were the largest seen in normal muscle. Hence in this typical 'mixed' muscle the superficial fast fibres have much larger end plates than the deep slow fibres. While it is true that the deep component of the tibialis anterior has motor end plates whose mean surface area is significantly smaller than those of the soleus, the mean surface area of the motor end plates in the superficial component is very significantly greater than those in the soleus. This is in contradiction to the observation of Coërs (1955) that the motor end plates in the tibialis anterior of the rabbit are smaller than those of the soleus. He did not separate the tibialis anterior muscle into superficial and deep components, and it appears likely that what Coërs had estimated were the motor end plates in the deep component.

Mann & Salafsky (1970) reported that although there was no difference in the end plate areas of the tibialis anterior and soleus at birth, from three weeks of age onwards the end plates of the tibialis anterior had a significantly greater area than those of the slow soleus. In their study also, the two components of the tibialis anterior were not differentiated and it is most likely that the end plates in the superficial component were studied.

Thus it appears that most workers have estimated the sizes of the motor end plates in either the superficial or the deep component and have taken this as being representative of the tibialis anterior muscle as a whole. On account of the highly significant difference ($P < 0.00001$) in the sizes of the end plates in the two components of the tibialis anterior muscle, they evidently should not be considered as a single functional unit, at least so far as the motor end plates are concerned. If a similar arrangement exists in man then it is likely that a disease process affecting neuromuscular junctions in the tibialis anterior muscle will affect the two components to different degrees and

hence a (superficial) biopsy taken from this muscle may not accurately reflect the changes taking place in the muscle as a whole. This could account for the low incidence of motor end plate abnormalities in biopsy specimens from patients with diseases like myasthenia gravis, where even in a severely affected case only 45 % of the end plates may appear abnormal (Coërs & Desmedt, 1958), possibly because the deeper red fibres, which are usually not removed in a biopsy, are affected earlier and to a more severe extent than the pale superficial fibres.

Susheela, Hudgson & Walton (1968) have reported that even muscles like the gastrocnemius have a component close to the bone surface which is almost wholly constituted of type I ('slow') fibres, while in the more superficial part of the muscle the type II ('fast') fibres predominate. In the rabbit gastrocnemius such a distinction is not at all clear, and no attempt was made to establish the existence of two separate components in this muscle. I am unable to account for this superficial layer of pale muscle fibres in the extensor muscles of the hind limb, or for the large end plates in the superficial ('fast') component of the tibialis anterior.

The large size of the motor end plates in the slow twitch muscles can, however, be related to their function. The slow twitch muscles are essentially postural muscles, exhibiting continuous activity (Vrbova, 1963). Because of the continuous barrage of impulses, the slow motoneurons presumably produce a greater aggregate number of impulses over long periods of time than the phasic motoneurons (Fischbach & Robbins, 1969) and hence the large motor end plate area of these continuously active slow muscles may facilitate the release of transmitter, for Kuno, Turkanis & Weakly (1971) showed that the amount of transmitter released following nerve stimulation is related to the size of the motor end plate. In short, the large size of the motor end plates of the red muscles may relate to a greater overall functional activity.

Buller, Eccles & Eccles (1960) postulated a chemical substance travelling down the motoneuron and influencing the mechanical responses of the muscle fibre, making it either fast or slow. No such factor has been identified as yet, though Drachman (1967) considers that acetylcholine is the trophic factor. The axoplasmic migration of substances from the cell body towards the periphery in sensory and motor nerves has been demonstrated (Miani, 1963; McEwen & Grafstein, 1968; Lasek, 1968; Ochs, Sabri & Johnson, 1969) and the passage of neuroplasmic components across the myoneural junction has also been demonstrated (Korr, Wilkinson & Chornock, 1967). Hence the existence of a trophic nerve agent cannot be ruled out. If there is a trophic factor governing the differentiation of muscle fibres and regulating their speeds of contraction, then the size of the motor end plate may be crucial because the passage of the trophic factor should undoubtedly be influenced by the area of contact which the motoneuron establishes with the muscle fibre.

SUMMARY

1. The surface areas of vitally stained motor end plates in 'fast' and 'slow' twitch skeletal muscles of the rabbit hind limb were measured.
2. The 'slow' soleus muscle contained motor end plates which had a mean surface area significantly larger than those in the gastrocnemius and flexor digitorum longus.

3. The mean surface area of the motor end plates in the lateral head of gastrocnemius was significantly smaller than those in the slower medial head.

4. In general, therefore, there appeared to be a relationship between the speed of contraction of a muscle and the size of its end plates. An exception, however, was the tibialis anterior muscle.

5. The motor end plates in the superficial ('fast') component of the tibialis anterior were significantly larger in size than those in the deep ('slow') component. This is in contrast to the findings of the typical fast and slow twitch muscles.

6. If a trophic factor regulating the speed of contraction of skeletal muscle really exists, then its secretion should be influenced by the area of the motor end plate: and it is suggested that the large end plates of the red muscles may be an adaptation to their function of continuous slow activity.

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