matters arising

Repression of synaptic efficacy in frog skeletal muscle

GRINNELL, Rheuben and Letinsky[1] show convincingly that when the transplanted frog sartorius muscle is innervated by two foreign nerves, control of the muscle fibres may be shared between the two, one nerve being effective at transmission the other eliciting only subthreshold junction potentials of low quantal content. They suggest this depends on a mutual competition between nerves similar to that invoked to explain selective reinnervation of urodele muscle[2,3].

I wonder whether this pattern of innervation is simply a property of the frog sartorius muscle. Luff and Proske[4] have found using tension measurements, that there is very little overlap between motor units when they measure twitch tension but extensive overlap (50% or more) when tetani were used. Furthermore, motor units in which the twitch tension fluctuated from trial to trial required high rates of stimulation to achieve peak tetanic tension and had low twitch tetanus ratios. This suggests that normally many muscle fibres are supplied with junctions which have a low safety factor for neuromuscular transmission. Presumably those fibres are also supplied with a safe junction from another motoneurone. Since there are only about 12 motor axons supplying the sartorius it seems unlikely, although not impossible, that the reduced efficacy of some of the junctions depends on an embryological matching process such as that postulated to account for selective innervation of muscles after nerve regeneration in urodeles. Rather it may be because sartorius muscle fibres tend to maintain one neuromuscular junction at high efficacy and reduce the efficacy of others on the same fibre, regardless of the embryological origin of the synapses, an interesting developmental mechanism in its own right but not necessarily related to the formation of specific connections. One could answer the question by finding out whether in the preparation used by Grinnell *et al.* there were unequal connections made by different motoneurones within the two populations of foreign nerve fibres. The test would be to split one of the foreign nerves and see if there was evidence for competition between different populations of fibres in one nerve, as there is between the populations of fibres of the two foreign nerves.

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1 Grinnell, A. D., Rheuben, Mary B. & Letinsky M. S. *Nature* **265**, 368–370 (1976).
2 Yip, J. W. & Dennis, M. J. *Nature* **260**, 350–352 (1976).
3 Genat, B. R. & Mark, R. F. *Phil. Trans. R. Soc.* **B278**, 335–346 (1977).
4 Luff, A. R. & Proske, U. *J. Physiol, Lond.* **258**, 673–685 (1976).

GRINNELL *et al.* REPLY—Mark rightly asks the question: If competition between two different nerves reduces the effectiveness of many terminals, as we report, then should not the same phenomenon occur between axons of the same nerve? If so, might this not be a normal phenomenon whereby each fibre acquires (relatively) exclusive innervation by one axon? This was an obvious question to us as well, and we have done some experiments of the type he suggests.

Our results indicate that there is competition between axons of the same nerve, but much less than between axons of two different foreign nerves. When a single nerve (normal or reinnervated) is split into two or more segments, with one to three functional motor units in each, there is little if any twitch overlap (usually 5% or less), but up to 25% tetanus overlap. This implies the existence, in *Rana catesbeiana* as in *Litoria aurea*[1], of some subthreshold polyneuronal innervation, and leads us to agree with Mark that the competition we have demonstrated between pairs of foreign nerves may be a special case of similar competition between axons of a single nerve. In our experiments there is a sharp quantitative difference between competition in the two cases, however, and probably a qualitative difference. The tension overlap we find between axons in singly innervated muscles does not approach that reported by Luff and Proske[1]. The explanation for this difference may be one of species or technique. It is noteworthy, for example, that Luff and Proske's Ringer apparently contained only 1.08 mM Ca^{2+} compared with the 1.8 mM in our amphibian Ringer and that used by most other investigators. In four experiments in our laboratory, shifting a normal sartorius preparation from 1.8 mM Ca^{2+} to 1.08 mM Ca^{2+} Ringer caused no change in 50 per s tetanus tension, but a reversible 53 ± 28% drop in twitch tension, suggesting that quantal content in a high proportion of junctions can be reduced below threshold by that reduction in Ca^{2+} concentration. This could also help account for Luff and Proske's low twitch-tetanus ratios. Although we find a few subthreshold junctions in singly reinnervated muscles, normally the quantal content is close to threshold, never as low as is commonly found in doubly innervated muscles (under 10). Moreover, the average twitch tension developed by simultaneous stimulation of both of two foreign nerves is conspicuously less (in percentage of fibres twitching) than that elicited by stimulation of the nerve in singly re-innervated muscles. This implies either fewer fibres are innervated, or that there is a greater number of subthreshold junctions where two different nerves are present rather than only one. Since we normally find that either nerve when tetanised can drive almost 100% of the muscle, most of the junctions formed by each must be subthreshold[2].

The competition we describe between the foreign somatic motor nerves is nonspecific; neither has an apparent innate advantage. Thus we do not propose this as a direct explanation for the reported instances of specific repression of one nerve by another. On the other hand, we do see some distinction between axons of two different nerves, compared with those of a single nerve. Moreover, the competition occurs in a muscle with focal rather than distributed endings, and hence is amenable to direct study of single 'repressed' endings. It is our hope that an understanding of this interaction may provide important insights into more specific forms of interaction.

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1 Luff, A. R. & Proske, U. *J. Physiol., Lond.* 258 673–684 (1976).
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