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Presynaptic and postsynaptic competition in models for the development of neuromuscular connections

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Abstract. In the establishment of connections between nerve and muscle there is an initial stage when each muscle fibre is innervated by several different motor axons. Withdrawal of connections then takes place until each fibre has contact from just a single axon. The evidence suggests that the withdrawal process involves competition between nerve terminals. We examine in formal models several types of competitive mechanism that have been proposed for this phenomenon. We show that a model which combines competition for a presynaptic resource with competition for a postsynaptic resource is superior to others. This model accounts for many anatomical and physiological findings and has a biologically plausible implementation. Intrinsic withdrawal appears to be a side effect of the competitive mechanism rather than a separate non-competitive feature. The model's capabilities are confirmed by theoretical analysis and full scale computer simulations.

1 Introduction

In several parts of the vertebrate nervous system the development of nerve connections involves an initial stage of superinnervation followed by withdrawal of axon terminals until just one contact per target cell remains. The best studied case is the innervation of mammalian skeletal muscle by its motor nerve (Redfern 1970; Jansen and Fladby 1990). During prenatal development, the axons of the motor neurons grow towards their target muscle and near the muscle each axon arborizes to innervate a large number of muscle fibres. At birth each muscle fibre is contacted by terminals from several different motor neurons. During the first few weeks after birth, axons lose some of their terminals until each muscle endplate is innervated by a single axon (Fig. 1). A similar pattern of events is found during reinnervation of adult muscle after injury to the motor nerve (McArdle 1975).

The mechanisms for the withdrawal of connections in the neuromuscular system are still largely unknown. The loss of the extra terminals on each muscle fibre is not due to neuron death or addition of muscle fibres, since the numbers of neurons and fibres do not change significantly during the period in question. Microscopy studies show that terminals are actually resorbed into the motor neuron.

Several attempts have been made to construct formal models for the withdrawal of superinnervation during the development of neuromuscular connections. Since the actual biochemical reactions that control the development of the pattern of neuromuscular innervation are unknown, assumptions in the different models must be largely justified by their ability to reproduce the key experimental findings.

So far the main concern has been to formulate models that can achieve single innervation. At this level, the problem is that very different ideas can generate the same qualitative behavior. In order to reduce the number of possible mechanisms, it is important to design models that are applicable to a wider range of experimental findings. It is equally important to consider whether the models are biologically feasible in terms of the physical and chemical reactions they require.

In this paper we examine the basic ideas that have been proposed for the development of neuromuscular connections and we then investigate how far these ideas can be extended. Firstly, we review the anatomical and physiological findings concerning the withdrawal of superinnervation. In Sect. 3 we discuss evidence for competitive effects and we describe existing models employing competitive mechanisms. In Sect. 4 a model that combines two types of competition is described. Section 5 discusses how the implementation of this model can be improved and Sect. 6 reports the stability analysis that we performed on our own version of this model. In Sect. 7 we present computer simulations illustrating various properties of the model.

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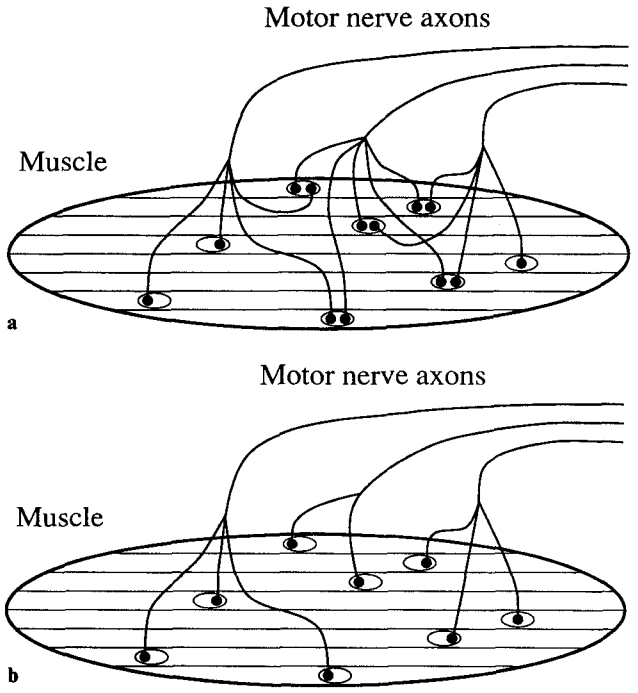


Fig. 1a, b. The muscle is innervated by a motor nerve containing three axons. Each muscle fibre has a single endplate. **a** Early stage. Several fibres are contacted by more than one axon. **b** Later stage. Polyinnervation has been withdrawn, leaving each endplate with a single terminal

2 Experimental findings

2.1 Normal development

In any one muscle the amount of initial innervation ranges from fibre to fibre with very few, if any, uninnervated muscle fibres. The average amount of initial innervation (the mean number of axons per endplate) varies from 2.7 in the rat lumbrical muscle (Betz et al. 1979) to 6.0 in the mouse soleus (Fladby and Jansen 1988). The extent of innervation can be also expressed in terms of the *motor unit size* (the number of fibres contacted by a given motor axon). The variability in size amongst the motor units of the rat soleus muscle decreases substantially during the elimination of polyinnervation. Brown et al. (1976) found that an initial 8-fold spread (defined as the ratio of the size of the largest motor unit to the smallest) was reduced to a 3-fold spread in the mature muscle. In contrast, no such reduction was seen in the rabbit soleus muscle (Gordon and van Essen 1981).

2.2 Partial denervation experiments and competition

In both neonates and adults, muscles can be partially denervated by removing some of the axons in the motor nerve. This experimental paradigm has been used to investigate the role of various possible mechanisms in the withdrawal of superinnervation.

Following neonatal partial denervation, the average size of the remaining motor units after withdrawal of superinnervation is larger than normal (Thompson and Jansen 1977; Fladby and Jansen 1987). This demonstrates that the fate of a terminal depends on the presence of other terminals, which leads to the idea of a competitive mechanism: terminals from different axons compete for the same endplate.

However, competition alone may not account for all findings. Partial denervation experiments where all but a single motor axon were removed were used to investigate whether terminals at singly innervated endplates could be withdrawn, even in the absence of competition from other terminals. One set of experiments on neonatal rat lumbrical muscle (Betz et al. 1980) indicated no withdrawal under these conditions. On the contrary, in experiments involving partial denervation of the mouse soleus, Fladby and Jansen (1987) calculated that the number of innervated muscle fibres in the adult was less than the number innervated initially; some terminals must have withdrawn to leave uninnervated endplates. This has led some authors to suggest the separate mechanism of *intrinsic withdrawal*, by which a certain small number of the initial set of connections made by each motor axon are always withdrawn (Thompson and Jansen 1977; Fladby and Jansen 1987).

2.3 Sprouting and reinnervation

In the adult, the existence of uninnervated fibres provokes sprouting: new terminals grow from the terminal processes of existing axons to contact uninnervated fibres nearby.

If a large enough number of axons are left intact after partial denervation in the adult, the extent of sprouting from these axons will be enough to cover the entire muscle. This is shown by experiments on the rat lumbrical muscle, which is innervated by the sural nerve and the lateral plantar nerve. Following damage to the sural nerve, the lateral plantar nerve expands its territory to innervate the entire muscle. On subsequent reinnervation, the sural nerve can reoccupy some of the muscle fibres that became occupied by the intact nerve (Ribchester and Taxt 1983).

3 Mechanisms and models

In this paper we examine the basic mechanism of competition whereby the number and distribution of contacts made by each motor neuron drive the withdrawal of superinnervation. We do not consider the additional effects of activity (Thompson 1985), type matching (Fladby and Jansen, 1988) and topographical arrangement (Laskowski and Sanes 1988).

Models invoking competitive mechanisms are well known in theoretical neuroscience. The most common types are those involving competition between the various terminals of each presynaptic neuron (Willshaw and von der Malsburg 1979) or between the terminals connecting each postsynaptic neuron (von der Malsburg 1973). In most cases, the action of such mechanisms has been expressed as the conservation of some

measure of the total amount of synaptic strength available to the neuron in question. How such a conservation process is implemented is not usually discussed. Since in this case the presynaptic elements (motor axons) compete for control of the postsynaptic element (endplate), the competition must be at the postsynaptic site.

3.1 A model relying on competition for a postsynaptic resource

The idea behind the model proposed by Gouzé et al. (1983) is that the terminals at each endplate compete for a share of a certain substance, assumed to be available in a limited amount at each endplate. This substance is gradually transferred to the terminals at that endplate, where it is converted into *survival factor*. The rate of transfer to a particular terminal is assumed to be a steeply rising function of the amount of postsynaptic substance already taken up by the terminal. The small random differences in the initial amount of factor assigned to terminals become magnified by this process until just one terminal survives.

According to this model, the elimination of terminals at each endplate occurs independently of the elimination at other endplates. Therefore the model can not explain why large motor units reduce in size more than smaller ones, and how one nerve can regain territory at the expense of another one. Although a model relying on the mechanism of competition for a postsynaptic resource alone can account for the establishment of single innervation, it cannot account for a substantial number of related experimental findings.

3.2 Importance of a limited presynaptic capacity

Many of the experimental results indicate that motor neurons each have the same, limited capacity to maintain terminals.

Removal of most of the motor units in the neonatal mouse soleus leads to incomplete innervation in the adult muscle (Fladby and Jansen 1987). Under these conditions the average motor unit size is found to be independent of the remaining number of motor units. This suggests that each neuron can maintain only a limited *number* of terminals. If presynaptic capacity were limited, the terminals of larger motor units would be weaker and therefore less competitive than those of smaller motor units. This would account for the reduction in spread of motor unit sizes during withdrawal in rat soleus. It would also be consistent with the finding that during reinnervation of partially denervated muscle by the severed nerve, the regenerating axons are able to take over control of some of the endplates from the intact neurons that had expanded their territory.

Gordon and van Essen (1981) found no reduction in the variability of motor unit sizes in the rabbit soleus. However, rabbit soleus is a very inhomogeneous muscle, whereas rat soleus contains predominantly slow fibres. If motor neurons innervate almost exclusively fibres of their own type (slow or fast), there may be separate reductions in the spread of motor unit sizes within the slow and fast parts of the muscle, without an overall reduction being seen.

3.3 Model using competition for a presynaptic resource

Willshaw's earlier model (1981) was intended to represent the idea of O'Brien et al. (1978), who proposed that each terminal releases a digestive enzyme into its endplate region, which decreases the capacity of all the terminals at the endplate to survive. In this model there is also counterbalancing growth of terminals such that the total "survival strength" assigned to the terminals of each motor neuron stays at a fixed level. In this way a presynaptic conservation rule (Lichtman 1977) is employed.

A system functioning according to these rules will develop from an initial state of polyinnervation to a state where all fibres receive single innervation. This model also provides an explanation for the decrease in the range of motor unit sizes during development. Since the terminals of the larger motor units will be weaker and therefore less competitive than terminals from smaller units, the larger units will lose comparatively more terminals.

However, conservation of survival strength was introduced into the model as a mathematical constraint without specifying the biological processes that would implement it. This is a problem since the interactions producing growth and decay probably originate in very different biochemical processes (i.e. terminal growth and enzymatic digestion). These two factors must be finely balanced in order to ensure presynaptic conservation and ultimately the stability of the system. It is hard to see how the necessary coupling between these two very different mechanisms could be achieved.

One way around the problem of implementing a presynaptic sumrule is to assume that there is competition for a presynaptic *substance*, rather than using an abstract mathematical rule. The amount of presynaptic substance will be limited thus ensuring that the presynaptic sumrule is implemented in a biologically plausible way.

3.4 Combined presynaptic and postsynaptic conservation

Both presynaptic and postsynaptic competition have desirable effects. Postsynaptic competition is necessary to achieve single innervation and presynaptic competition gives an account of many experimental findings.

This leads naturally to the idea of combining the two types of competition in a single model, as proposed by Bennett and Robinson (1989). We call this type of model a *dual constraint model*, or DCM.

4 Bennett and Robinson's dual constraint model

We first describe this model, using our own notation. In the next two sections we show how we have developed the model.

There are N motor neurons and M muscle fibres. A particular motor neuron is indexed by n, a muscle fibre by m and a terminal by nm. The key dependent variables are the quantities A_n, A_{nm}, B_m and C_{nm}, defined below, which are all functions of time. Since not all neurons have terminals at all muscle fibres, the sums taken over neurons and muscle fibres are only over the terms for which terminals exist.

In this model a reversible reaction between the presynaptic substance A and postsynaptic substance B produces binding complexes C. These are essential to the maintenance of terminals; the size of terminal nm is assumed to be directly proportional to C_{nm}.

The reactions take place in the synaptic cleft, where presynaptic molecules are found in the terminal membranes and postsynaptic molecules are located in the endplate membrane. The reversible chemical reaction takes one molecule of each of A and B to produce one of C

$$A_{nm} + B_m \rightleftharpoons C_{nm} . \tag{1}$$

Each motor neuron n has a fixed amount A_0 of presynaptic substance A available to it. Molecules of A can be located either in the cell soma, in amount A_n, or in one of the terminals of the neuron. In terminal nm, A can either be unbound in the terminal membrane, in amount A_{nm}, or bound, in amount C_{nm}. The conservation equation for A is

$$A_0 = A_n + \sum_{j=1}^{M} A_{nj} + \sum_{j=1}^{M} C_{nj} . \tag{2}$$

Bennett and Robinson assumed that the amount of unbound presynaptic substance in a terminal is proportional to *(i)* the size of the terminal C_{nm} and *(ii)* the amount of presynaptic substance in the cell soma A_n, yielding

$$A_{nm} = KC_{nm}A_n , \tag{3}$$

where K is a constant.

Each endplate m has a fixed amount B_0 of postsynaptic substance B available to it. Molecules of B can either be unbound in the endplate membrane, in amount B_m, or bound, in amount C_{nm}, at the site. The conservation equation for B is

$$B_0 = B_m + \sum_{i=1}^{N} C_{im} . \tag{4}$$

4.1 Reaction kinetics

The reversible chemical reaction between A_{nm} and B_m involves a forward and a backward reaction. The forward reaction rate is taken to be proportional to the product of the amounts of the presynaptic substance A_{nm}, the postsynaptic substance B_m and a fixed power μ of the amount of binding complex C_{nm} that are locally available. The backward reaction rate is taken to be proportional to the amount of binding complex, C_{nm}.

$$\frac{dC_{nm}}{dt} = K_1 A_{nm} B_m C_{nm}^{\mu} - K_2 C_{nm} , \tag{5}$$

where K_1 and K_2 are rate constants. The justification for including a term involving C_{nm}^{μ} is that electrical activity in the endplate region could produce electromigration of molecules of B in the endpoint membrane. The result is that the larger terminals will have an ability to attract the postsynaptic molecules to the endplate region immediately under the terminal – thus favouring the forward reaction rate at these terminals. This is implemented by fixing μ at a small positive value.

Using (2)–(4) to express B_m and A_{nm} in terms of C_{nm}

$$A_{nm} = K \frac{A_0 - \sum_{j=1}^{M} C_{nj}}{1 + K \sum_{j=1}^{M} C_{nj}} C_{nm} \quad \text{and} \quad B_m = B_0 - \sum_{i=1}^{N} C_{im} . \tag{6}$$

Introducing these expressions for A_{nm} and B_m into (5) gives a set of first-order differential equations for how C_{nm} changes over time.

4.2 Simulation results

Bennett and Robinson chose the value of the ratio A_0/B_0 so as to make the total amount of presynaptic substance equal to the total amount of postsynaptic substance; i.e., $A_0/B_0 = M/N$.

In their numerical calculations, a set of terminals was chosen at random and each was given the same small amount of the binding complex C. Numerical solutions of the set of differential equations for C_{nm} as defined by (5) and (6) demonstrated that during the initial phase all terminals grew, but eventually all but a single terminal at each endplate retracted. In most cases, a state of single innervation was reached.

5 Issues of implementation in dual constraint models

5.1 Transport of A

Bennett and Robinson do not discuss what processes could give rise to the distribution of A given by (3). We suggest a scheme of transport mechanisms that should be easy to implement.

A terminal that is growing rapidly must have a net flow of A to the terminal, whereas a terminal that is shrinking will have a net flow *from* the terminal; both anterograde and retrograde transport are required. We use the familiar physical principle that the rate of transport is proportional to the concentration of substance at the sending site.

The rate of *anterograde* transport down the axon of the motor neuron is assumed to be proportional to the concentration of presynaptic substance in the cell body. Since the size of the cell body is constant, this concentration is proportional to the number of presynaptic molecules there, A_n. We suggest the simplifying approximation that when the molecules reach the arborisation of the axon, this anterograde flow becomes divided evenly between the ν_n terminals of the neuron. This will probably require that all the terminal sprouts arborize from the same point and have similar characteristics. This division of flow should be simple to implement since it does not require a sophisticated computation – at the point of

arborisation no knowledge is needed about the size of the terminal at the end of each axon branch relative to the number and size of the other terminals.

The rate of *retrograde* transport from each terminal is also taken to be proportional to the concentration of presynaptic molecules. In the two-dimensional terminal membrane, this concentration is proportional to A_{nm}/C_{nm}, given that the size (area) of a terminal is proportional to C_{nm}. This equation could give rise to difficulties if C_{nm} were allowed have values close to zero. This situation will never arise however since very small terminals are removed explicitly (see below).

We can then form a differential equation for the transport of A

$$\frac{dA_{nm}}{dt} = \gamma \frac{A_n}{v_n} - \delta \frac{A_{nm}}{C_{nm}}, \tag{7}$$

where γ and δ are rate constants.

Equilibrium is achieved when

$$\frac{dA_{nm}}{dt} = 0 \Leftrightarrow A_{nm} = \frac{\gamma}{\delta v_n} A_n C_{nm}. \tag{8}$$

If the transport rate constants (γ and δ) are large, the system will settle quickly towards equilibrium. During withdrawal the equilibrium point changes continuously (since it depends on $A_n C_{nm}$), and so large values of γ and δ will cause the system to be close to equilibrium at all times.

By equating the constant K of (3) used in the original model of Bennett and Robinson with $\gamma/\delta v_n$ of (8), it can be seen that Bennett and Robinson's equation (3) corresponds to the steady state in our interpretation. An important difference is that the new term has a dependency on v_n.

5.2 Clarification of the justification of reaction dynamics

The account of the chemical reaction rates given by Bennett and Robinson does not justify very clearly the relation between terminal size and reaction rate. We offer the following interpretation.

Molecules of A and B in the pre- and postsynaptic membranes move freely about in the membranes. The rate of the forward reaction is taken to be proportional to the probability that a molecule of A and one of B collide. This probability is proportional to the product of the concentrations of A and B and the size of the reaction surface (the terminal membrane area) which is proportional to C.

In order to achieve single innervation it is important to favour larger terminals over smaller ones. According to (5), the forward reaction rate has a dependency on C_{nm}. We introduce a linear dependency, to give

$$\frac{dC_{nm}}{dt} = \alpha A_{nm} B_m C_{nm} - \beta C_{nm}. \tag{9}$$

The backward reaction rate is assumed to be proportional to the amount C_{nm} of binding complexes that are available to break up. We find it difficult to justify inclusion of the dependency of the forward reaction rate on C_{nm} beyond saying that a way of favouring larger terminals (i.e. terminals that possess a large amount of C_{nm}) over smaller ones is needed to achieve single innervation. It is not clear to us what the physical mechanisms underlying this dependency could be. As mentioned in Sect. 4.2, Bennett and Robinson proposed electromigration.

5.3 Explicit withdrawal of small terminals

Terminals that possess a very small amount of C_{nm} are considered by Bennett and Robinson to be eliminated. This elimination does not have any consequence for their equations.

However, in our scheme the substance A must be transported only to the terminals that do exist, and so explicit withdrawal of terminals is required. We regard any terminal for which the value of C_{nm} has fallen below a small threshold value θ as being withdrawn. This is done by removing the equations for terminal nm and decreasing the corresponding v_n by one. The tiny amounts of A and B held by the terminal are resorbed into the motor neuron and the muscle endplate respectively.

The model proposed by Willshaw (1981) has been criticized (Gouzé et al. 1983) for the discontinuity introduced when a terminal withdraws. In our model a similar discontinuity arises (stepwise decrement of v_n), but here it is supported by the underlying implementation.

6 Mathematical analysis

The development of connections according to the revised model of Bennett and Robinson is specified by two sets of first-order differential equations. Using equations (2), (4), (7), (9), for terminal nm these are

$$\frac{dC_{nm}}{dt} = \alpha A_{nm}\left(B_0 - \sum_{i=1}^{N} C_{im}\right)C_{nm} - \beta C_{nm}, \tag{10}$$

$$\frac{dA_{nm}}{dt} = \frac{\gamma}{v_n}\left(A_0 - \sum_{j=1}^{M} A_{nj} - \sum_{j=1}^{M} C_{nj}\right) - \delta \frac{A_{nm}}{C_{nm}}. \tag{11}$$

These equations are difficult to handle analytically. Because of the nonlinearity of the equations we have not been able to solve them in the general case. Our computer simulations (Sect. 7) suggest that states of polyinnervation are unstable, but we have not been able to prove this analytically. However, analysis of the single innervation case is possible.

6.1 Stability of the single innervation state

Using perturbation analysis, we derive the conditions under which single innervation is a stable state. We test whether a given equilibrium state is stable against noise (with no DC component) in the values of C_{nm} and A_{nm}.

Let $\tilde{C}_{nm}$ and $\tilde{A}_{nm}$ denote an equilibrium state. Further let ϕ_{nm} and ψ_{nm} denote small perturbations to an equilibrium state given by

$$\frac{d\tilde{C}_{nm}}{dt} = 0 \qquad C_{nm} = \tilde{C}_{nm} + \phi_{nm}, \tag{12}$$

$$\frac{d\tilde{A}_{nm}}{dt} = 0 \qquad A_{nm} = \tilde{A}_{nm} + \psi_{nm}. \tag{13}$$

The first order (linear) Taylor expansion about the equilibrium point is given by

$$\frac{\mathrm{d}\phi_{nm}}{\mathrm{d}t} = \alpha(\tilde{A}_{nm} + \psi_{nm})\left[B_0 - \sum_{i=1}^{N} \tilde{C}_{im} - \sum_{i=1}^{N} \phi_{im}\right] \times (\tilde{C}_{nm} + \phi_{nm}) - \beta(\tilde{C}_{nm} + \phi_{nm})$$

$$\simeq \alpha\tilde{C}_{nm}\left(\left[B_0 - \sum_{i=1}^{N} \tilde{C}_{im}\right]\psi_{nm} - \tilde{A}_{nm}\sum_{i=1}^{N} \phi_{im}\right), \quad (14)$$

$$\frac{\mathrm{d}\psi_{nm}}{\mathrm{d}t} = \frac{\gamma}{v_n}\left[A_0 - \sum_{j=1}^{M} \tilde{A}_{nj} - \sum_{j=1}^{M} \psi_{nj} - \sum_{j=1}^{M} \tilde{C}_{nj} - \sum_{j=1}^{M} \phi_{nj}\right] - \delta\frac{\tilde{A}_{nm} + \psi_{nm}}{\tilde{C}_{nm} + \phi_{nm}} \simeq \frac{\delta}{\tilde{C}_{nm}^2}(\tilde{A}_{nm}\phi_{nm} - \tilde{C}_{nm}\psi_{nm}) - \frac{\gamma}{v_n}\left[\sum_{j=1}^{M} \phi_{nj} + \sum_{j=1}^{M} \psi_{nj}\right]. \quad (15)$$

Writing this in matrix form by lining up all ϕ_{nm}'s and ψ_{nm}'s to form a vector $\mathbf{x}$, we have

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathscr{W}\mathbf{x} \quad \text{with solutions} \quad \mathbf{x} = \sum_i k_i \mathbf{u}_i \exp(\gamma_i t), \quad (16)$$

where k_i are constants, $\mathbf{u}_i$ are eigenvectors of $\mathscr{W}$ and λ_i the corresponding eigenvalues. This shows that upon perturbation the system will settle back towards the equilibrium point $\mathbf{x} = \mathbf{0}$ if all $\Re(\lambda_i) < 0$ (where $\Re$ denotes the real part). Thus, the equilibrium point corresponds to a stable point if all eigenvalues λ_i have negative real parts.

In the single innervation case all motor units are completely independent. Therefore, let us look at a single motor unit. For any one of its terminals nm in the single innervation state, $\sum_{i=1}^{N} C_{im} = C_{nm}$. Introducing this in (10) and solving (10) and (11) for $\tilde{C}_{nm}$ and $\tilde{A}_{nm}$ we deduce that all $\tilde{C}_{nm}$ and all $\tilde{A}_{nm}$ belonging to the same motor unit will take the same value – called $\tilde{C}$ and $\tilde{A}$ respectively. We now make a small scale version of vector $\mathbf{x}$, by including only ϕ's and ψ's corresponding to actual terminals of the neuron. The neuron in question has v terminals, so

$$\mathbf{x} = (\phi_1, \phi_2, \ldots, \phi_v, \psi_1, \psi_2, \ldots, \psi_v)^T. \quad (17)$$

The eigenvalues λ_i of the corresponding matrix $\mathscr{W}$ are solutions of $\det(\mathscr{W} - \lambda\mathscr{I}) = 0$ where $\mathscr{I}$ is the unit matrix of size $2v$. The general form of this equation is

$$\begin{vmatrix} a-\lambda & 0 & \ldots & 0 & 0 & b & 0 & \ldots & 0 & 0 \\ 0 & a-\lambda & \ldots & 0 & 0 & 0 & b & \ldots & 0 & 0 \\ \vdots & \vdots & & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ 0 & 0 & \ldots & a-\lambda & 0 & 0 & 0 & \ldots & b & 0 \\ 0 & 0 & \ldots & 0 & a-\lambda & 0 & 0 & \ldots & 0 & b \\ c+f & f & \ldots & f & f & g-\lambda & f & \ldots & f & f \\ f & c+f & \ldots & f & f & f & g-\lambda & \ldots & f & f \\ \vdots & \vdots & & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ f & f & \ldots & c+f & f & f & f & \ldots & g-\lambda & f \\ f & f & \ldots & f & c+f & f & f & \ldots & f & g-\lambda \end{vmatrix} = 0, \quad (18)$$

where

$$a = -\alpha\tilde{C}\tilde{A} \quad b = \alpha\tilde{C}[B_0 - \tilde{C}] \quad c = \frac{\delta\tilde{A}}{\tilde{C}^2}$$

$$d = -\frac{\delta}{\tilde{C}} \quad f = -\frac{\gamma}{v} \quad g = d + f. \quad (19)$$

The matrix equation (18) can by the following operations

$$\text{for } i = 1 \ldots v: \mathrm{row}_{v+i} := \mathrm{row}_{v+i} - \frac{d-\lambda}{b}\mathrm{row}_i - \frac{f}{b}\sum_{j=1}^{v}\mathrm{row}_j,$$

$$\text{for } i = 2 \ldots v: \mathrm{row}_{v+i} := \mathrm{row}_{v+i} - \mathrm{row}_{v+1}$$

$$\mathrm{col}_1 := \mathrm{col}_1 + \sum_{j=2}^{v}\mathrm{col}_j,$$

be reduced to

$$[\lambda^2 - (a+d)\lambda + ad - cb]^{v-1}[\lambda^2 - (a+d+vf)\lambda - ad - cb + vfb - vfa] = 0,$$

The roots and the conditions that they have negative real parts are

$$\lambda = \tfrac{1}{2}[a + d \pm \sqrt{(a+d)^2 + 4(cb - ad)}] < 0 \Rightarrow cb < ad,$$

$$\lambda = \tfrac{1}{2}[a + d + vf \pm \sqrt{(a+d+vf)^2 + 4(cb - ad + vf(b-a))}] < 0$$

$$\Rightarrow cb + vfb < ad + vfa,$$

By reintroducing (19) it is found that the sufficient and necessary condition that these equations only have negative roots is $\tilde{C} > B_0/2$. This same line of reasoning can be applied to all motor units with a similar result.

Since C_{nm} cannot exceed B_0 (from (4)) the limits on $\tilde{C}_{nm}$ for a stable single innervation state are

$$B_0/2 < \tilde{C}_{nm} < B_0. \quad (20)$$

Since $\tilde{C}_{nm}$ cannot be arbitrarily small it follows that there is an upper bound to the number of terminals that a given ratio A_0/B_0 will allow a motor neuron to support. Using (10)–(13) we obtain

$$A_0 - v(\tilde{A} + \tilde{C}) = \frac{\delta v}{\gamma}\frac{\tilde{A}}{\tilde{C}} \quad \text{and} \quad \tilde{A} = \frac{\beta}{\alpha}\frac{1}{B_0 - \tilde{C}}.$$

Combining these with $\tilde{C} > \frac{1}{2}B_0$ we arrive at an upper limit on the number of terminals

$$= \frac{\alpha\gamma A_0 \tilde{C}(B_0 - \tilde{C})}{\alpha\gamma\tilde{C}^2(B_0 - \tilde{C}) + \beta\gamma\tilde{C} + \beta\delta} < 2\frac{A_0}{B_0}. \quad (21)$$

6.2 The importance of the value of A_0/B_0

Our analysis shows that there is an upper limit on the number of terminals that can be supported by a neuron in the stable state of single innervation (21). This limit is proportional to the value of A_0/B_0. If therefore the value of A_0/B_0 is so low that the maximum number of terminals allowed is less than the number present initially, then terminals will retract – even in the absence of competition from other axons. Conversely, if the value A_0/B_0 is high enough, no intrinsic withdrawal may be needed.

This finding offers an explanation of the seemingly contradictory evidence obtained from different muscles concerning intrinsic withdrawal, by assuming that different muscles have different ratios of the initial number of terminals to the value of A_0/B_0 (Fig. 5).

Bennett and Robinson matched up the total amount of presynaptic and postsynaptic substance available, by setting $A_0/B_0 = M/N$. However, this seems a somewhat arbitrary decision. It seems unlikely that information about B_0, M and N would be available to each motor neuron to enable this equality to be maintained in, say, the partial denervation experiments, where the value of N is changed artificially. We chose the value of A_0/B_0 according to (21), to determine the maximum number of terminals per neuron. Intrinsic withdrawal will now occur only when the initial number of terminals violates (21). Note that elimination of terminals does not necessarily stop as soon as condition (21) is satisfied (see Sect. 7.3).

7 Simulations

Since our analysis was restricted to examining the stable state we needed to confirm that the model reproduces the entire process of withdrawal. To do this we employed computer simulations.

In the simulated system there are as many muscle fibres and motor neurons as are found in real muscles. Running simulations that model real muscles has the advantage that we can model cases that have been investigated experimentally and allows us to compare experimental findings with model behaviour with great ease.

Numerical solutions to the equations for A_{nm} and C_{nm} were obtained by approximating the differential equations with difference equations which are solved by an iterative method. Throughout these simulations the timestep value $\Delta t = 0.001$ days was used. The choice of Δt was justified by the finding that a doubling of Δt only had negligible influence (in the order of 0.1%) on simulation results.

These full scale simulations are compute intensive. Simulating the development over a few weeks of a muscle consisting of 3500 fibres innervated by 25 motor neurons with an initial degree of polyinnervation of 5 (as in Fig. 2), takes ~4 h on a SPARC (Sun 4/60) workstation.

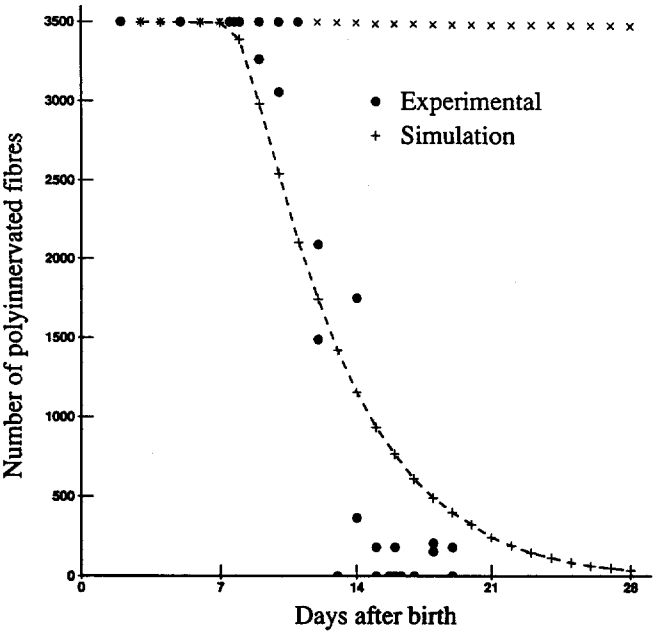


Fig. 2. The time course of the elimination of polyinnervation in rat soleus muscle. There are $N = 25$ motor neurons innervating $M = 3500$ muscle fibres. The amount of polyinnervation falls rapidly during the second and third week after birth. Polyinnervation is virtually extinct in animals older than 4 weeks. Also shown in the same graph (×) is the number of fibres in the simulation receiving *some* innervation, i.e. polyinnervation or single innervation. This shows that virtually all fibres that initially receive innervation are still innervated when polyinnervation has been withdrawn

7.1 Numerical values and initial innervation

When determining the initial pattern of innervation, there are two important distributions to consider.

Distribution of motor unit sizes. Should all neurons have an equal number of collaterals or should there be a wide spread in the initial motor sizes? For the rat soleus a wide initial spread was assumed. In other simulations all motor units approximately the same initial size.

Distribution of multiple innervation. How should the number of terminals per endplate be distributed? As far as we know, this question has not been addressed experimentally, but theoretical arguments support a random distribution (Willshaw 1981). We chose the number of terminals at each endplate from a normal distribution with a standard deviation less than the mean. This ensured that nearly all endplates were multiply innervated.

Once the terminals have been chosen, a starting value has to be assigned to A_{nm} and C_{nm} for each terminal nm. We set $A_{nm} = 0$ for all terminals and for

C_{nm} we used values drawn from a normal $\mathcal{N}(0.04, 0.005)$ distribution (i.e. mean 0.04 and standard deviation 0.005). It is important to introduce some kind of randomness into the system to break the symmetry. This is not a problem in the biological world since many factors introduce noise here. We set the threshold value at $\theta = 0.01$, but the exact value is unimportant since once terminals get below a certain size they will shrink continuously. Bennett and Robinson assumed that the initial value of C_{nm} should be identical for all terminals. In their system, symmetry is broken only by the spread in initial motor unit sizes. We think that our choice is biologically more reasonable.

In order to solve the equations numerically we need to specify the values of the constants and the ratio A_0/B_0. We fixed B_0 at 1 and determined the ratio A_0/B_0 by specifying A_0. The value of A_0/B_0 was chosen in accordance with physiological investigations of maximum motor unit size in each particular muscle type. The values of the rate constants α, β, γ and δ were adjusted empirically to mimic the time course of elimination found experimentally (Fig. 2). In all simulations the values were $\alpha = 45$, $\beta = 0.4$, $\gamma = 3.0$ and $\delta = 2.0$. The values have not been optimized in any systematic way, but our results do not seem to be very sensitive to exact parameter values.

We present simulations of 3 different muscles

The rat soleus muscle. This muscle typically has $N = 25$ neurons, $M = 3500$ muscle fibres and an initial degree of polyinnervation of 5. The initial distribution of multiple innervation was drawn from a normal $\mathcal{N}(5, 1)$ distribution. The distribution of motor unit sizes contained a large spread (Fig. 3).

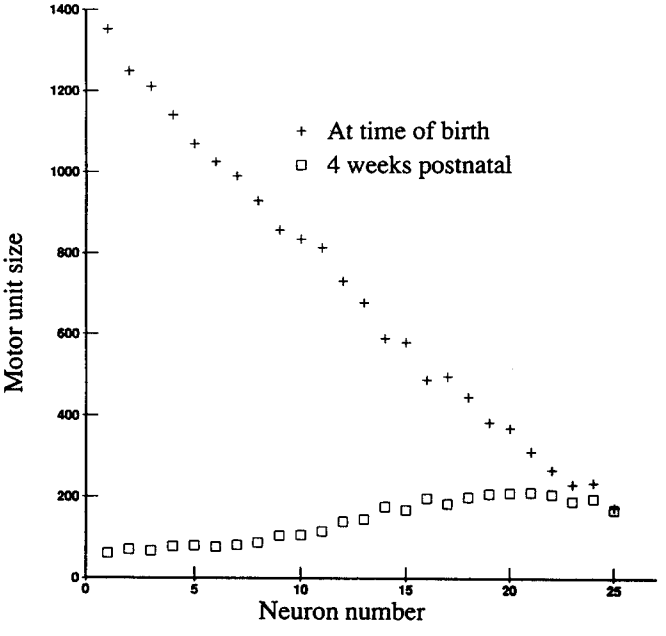


Fig. 3. Simulation of rat soleus muscle. Reduction in the spread of motor unit sizes during withdrawal of polyinnervation. There are $N = 25$ motor neurons and $M = 3500$ muscle fibres. The spread in motor unit sizes is reduced substantially from ~8 to ~3. This corresponds to the reduction found experimentally

The mouse soleus muscle. This muscle typically has $N = 20$ neurons, $M = 600$ muscle fibres and an initial degree of polyinnervation of 6. The initial distribution of multiple innervation was drawn from a $\mathcal{N}(6, 1)$ distribution. All motor units had roughly the same size initially.

The rat lumbrical muscle. This muscle typically has $N = 12$ motor neurons, $M = 600$ at time of birth but $M = 960$ in the mature muscle. The initial amount of polyinnervation is 2.7 typically and so the initial distribution of multiple innervation was drawn from a $\mathcal{N}(2.7, 0.5)$ distribution.

In all cases we assumed that competition begins 3 days after birth.

7.2 Elimination of polyinnervation in rat soleus

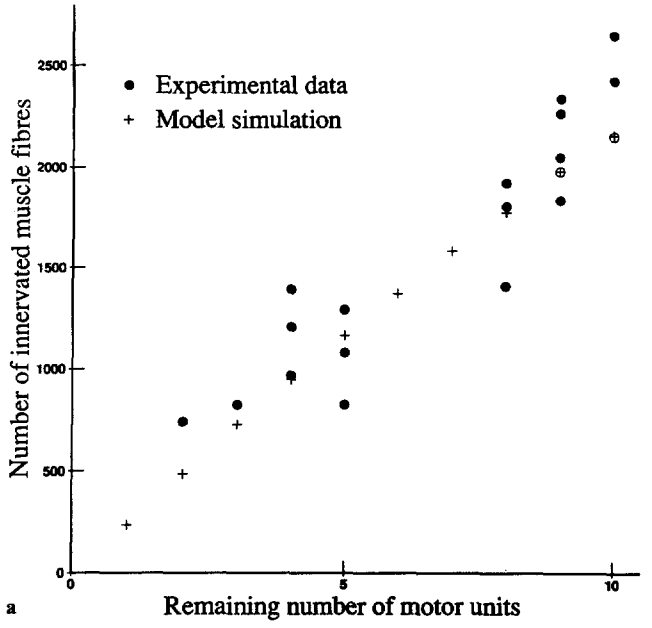
Figure 2 shows how the number of innervated fibres changes over time in a rat soleus muscle. Our simulation results are plotted together with experimental findings (Brown et al. 1976). The simulation results are close to the experimental data. In the same graph the number of fibres receiving *some* innervation is plotted. The fact that this curve stays very close to 3500 fibres show that only very few (if any) fibres become denervated.

Figure 3 shows the distribution of motor unit sizes at two stages of development, from the simulation shown in Fig. 2. At birth we introduced a spread (ratio of largest to smallest) of ~8, in line with findings of Brown et al. (1976). During the course of elimination there is a marked reduction in this spread, to ~3, as also found by these authors. Note that the motor units that are initially largest end up being very small. This is an effect of the presynaptic sum rule, seen also by Willshaw (1981).

7.3 Partial denervation experiments

Experiments on partial denervation of rat soleus (Thompson and Jansen 1977) and mouse soleus (Fladby and Jansen 1987) both concluded that the average size of the remaining motor units in the adult was independent of the number of remaining motor neurons, confirming that motor units have a limited maximum size. This size was smaller than the average size in neonatal muscle but larger than the normal adult size.

Figure 4a and Fig. 4b show simulation results together with experimental data. Figure 4a shows the situation for the rat soleus together with the findings of Thompson and Jansen (1977). Figure 4b shows the same for mouse soleus and the findings of Fladby and Jansen (1987). We used the experimental data to adjust our choice of the value of A_0/B_0. The values found were $A_0/B_0 = 175$ for the rat soleus muscle and $A_0/B_0 = 60$ for the mouse soleus.



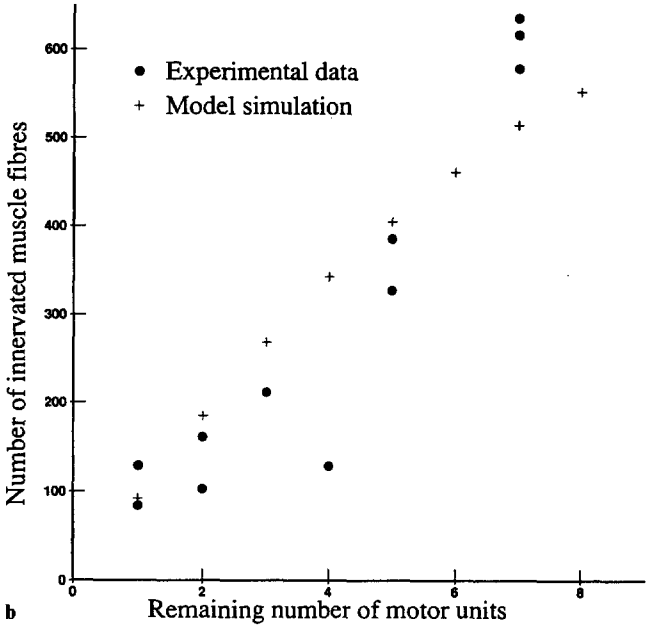


Fig. 4a, b. Degree of innervation in 6 week old muscles that were partially denervated at birth. The graphs show that there is an approximately proportional relationship between the number of motor units remaining after partial denervation and the number of fibres receiving innervation. This suggests that all motor units reach the same size. **a** Rat soleus has $M = 3500$ fibres and before partial denervation $N = 25$ motor neurons. Experimental findings from Thompson and Jansen (1977). **b** Mouse soleus has $M = 600$ fibres and before partial denervation $N = 20$ motor units. Experimental findings from Fladby and Jansen (1987)

To reach a value of A_0/B_0 for the rat lumbrical muscle we used a result by Ribchester (1987), who showed that if the muscle is innervated by 3 neurons or more it would be virtually completely innervated. The value of A_0/B_0 obtained through this observation is $A_0/B_0 = 200$.

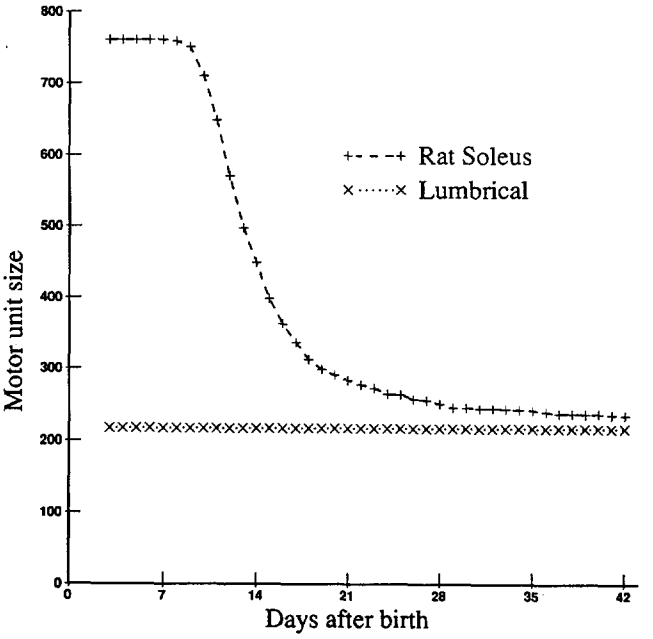


Fig. 5. Simulation of the development of motor unit size in the absence of competition from other motor neurons. Muscles were partially denervated at birth, so that only a single motor unit survived in each case. The effect of intrinsic withdrawal is seen for the rat soleus muscle only. At 6 weeks the size of the soleus motor unit is close to its final value

Conflicting findings have been obtained when addressing the question of whether the loss of terminals could be ascribed solely to competition or whether some kind of intrinsic withdrawal plays an important role. A convenient way of addressing the problem is to remove the competition at the endplate by dissecting out all but a single motor axon. In the absence of intrinsic withdrawal there should be no reduction of the motor unit size in these circumstances.

Figure 5 shows how the unit size of a single motor unit changes over time in the absence of competition from other motor units. Betz et al. (1980) found no evidence for intrinsic withdrawal in the rat lumbrical muscle. On the contrary, Fladby and Jansen (1987) found a reduction of motor unit sizes in rat soleus that could not be ascribed solely to the effects of competition between terminals at an endplate. Our findings show that the combined presynaptic and postsynaptic competition can account for these seemingly contradictory results.

The simulation results confirm our analytical results that withdrawal in the absence of competition from other motor units only takes place in certain cases depending on the value of A_0/B_0. It is important to note that there is no guarantee that a system that starts off with a large number of terminals will settle to a stable state as soon as (21) is satisfied. On the contrary, our simulations show that the system typically settles in a state with slightly fewer terminals than given in (21).

In Fig. 5, the final size of the single motor unit in the rat soleus is seen to be ~ 250, compared with an estimate from (21) of 350.

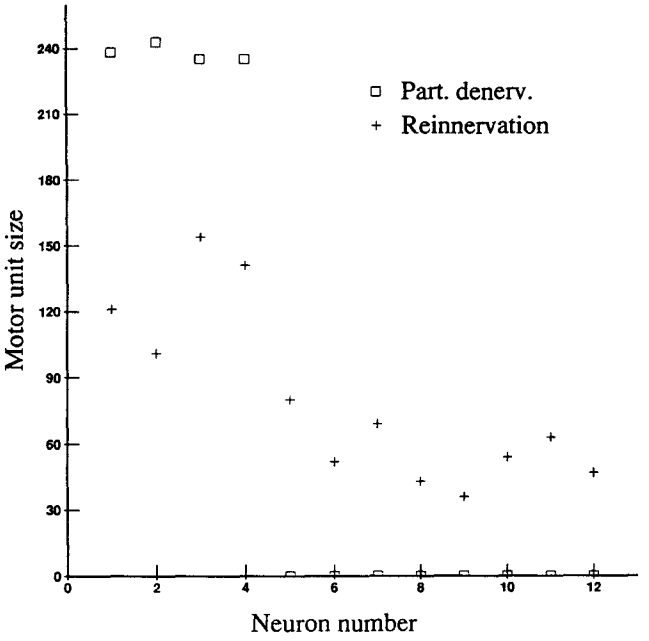


Fig. 6. Plasticity of the pattern of connections in the simulation of adult rat lumbrical muscle. The adult muscle was partially denervated so that only 4 motor neurons remained of the $N = 12$ that are normally present. These 4 motor units are assumed to have sprouted to innervate the entire muscle. Motor unit sizes are shown for the four intact units. When the injured motor neurons grew back, they recovered control over some muscle fibres, at the expense of the undamaged neurons. In the graph it is seen that all motor neurons have terminals, and motor units 1–4 have shrunk

7.4 Reinnervation of partially denervated muscle

Figure 6 refers to partial denervation of a mature lumbrical muscle. The simulation is designed to mimic experiments by (Ribchester 1988). Partial denervation was introduced by removing one (the lateral plantar nerve) of the two nerves supplying the muscle. The remaining neurons sprout and if there are at least three neurons left virtually all fibres get innervated. Around 2 weeks later the severed nerve returns to the muscle and competes with the remaining neurons. Ribchester reported that the reinnervating nerve was able to take over control of some muscle fibres from the remaining nerve. Our simulation shows the same ability. This is an important property of the model since it shows that the state of innervation is plastic even when single innervation has been reached.

8 Conclusion

Previous models for the elimination of superinnervation in developing muscle are based on certain mechanisms of competition. We have argued that issues of implementation (Willshaw 1981) and of the nature of the experimental results (Gouzé et al. 1983) make these mechanisms inadequate. The dual constraint model (DCM) due to Bennett and Robinson (1989) employs two types of competition: for a presynaptic substance and for a postsynaptic substance.

We resolved certain questions about implementation that Bennett and Robinson left outstanding, and then performed perturbation analysis on the set of equations defining the improved model. This enabled us to derive the result that for the state of single innervation to be stable, the number of terminals made by each motor neuron cannot exceed a certain figure.

This model mimics the experimental findings well and the underlying mechanisms are biologically plausible. Results from our analysis and our simulations showed that in all cases the system converged to a state of single innervation and the terminals of any particular motor neuron were all of the same size.

In some of Bennett and Robinson's smaller-scale simulations of their slightly different model, some endplates remained multiply innervated after many time steps. It is not clear to us whether these represent stable end-states and, if so, whether they are an artifact of the initial conditions that they chose.

In future work we will incorporate into the model the effect of activity and an explicit mechanism of sprouting. We will have to decide which of the biochemical reactions underlying the model should be made activity dependent. Sprouting can be thought of as the formation of new terminals into which small amounts of the presynaptic substance A that has not yet been converted to the binding complex C are released. Unlike other models (Gouzé et al. 1983), the underlying chemical reactions of DCM are reversible and so the stable endstate of single innervation may not remain stable once sprouting occurs.

Our most important finding is that there is a maximum number of terminals that a motor neuron can maintain, which is determined by the ratio of the amount A_0 of presynaptic substance available per motor neuron to the amount B_0 of postsynaptic substance per endplate. Bennett and Robinson chose the value of this ratio so as to match up the *total* amount of presynaptic substance to the total of postsynaptic substance. This is biologically unjustifiable and is not necessary computationally. If this ratio is very large, the presynaptic conservation rule becomes inoperative; if it is so small that the maximum number of terminals that a motor neuron can maintain is less than the initial amount of polyinnervation, then terminals will necessarily withdraw, even in the absence of competition from other terminals; i.e., there is intrinsic withdrawal. We set the value of A_0/B_0 in accord with the maximum number required by the results of neonatal partial denervation experiments.

Our analysis suggests that intrinsic withdrawal should not be regarded as a mechanism in its own right but rather a side effect of a general mechanism employed by the neuromuscular system to achieve similar motor unit sizes under a wide variety of conditions.

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References

Bennett MR, Robinson J (1989) Growth and elimination of nerve terminals during polyneuronal innervation of muscle cells: a trophic hypothesis. Proc R Soc Lond [Biol] 235: 299–320.

Betz WJ, Caldwell JH, Ribchester RR (1979) The size of motor units during post-natal development of rat lumbrical muscle. J Physiol (London) 297:463–478

Betz WJ, Caldwell JH, Ribchester RR (1980) The effects of partial denervation at birth on the development of muscle fibres and motor units in rat lumbrical muscle. J Physiol (London) 303:265–279

Brown MC, Jansen JKS, van Essen DC (1976) Polyneuronal innervation of skeletal muscle in new-born rats and its elimination during maturation. J Physiol (London) 261:387–422

Fladby T, Jansen JKS (1987) Postnatal loss of synaptic terminals in the partially denervated mouse soleus muscle. Acta Physiol Scand 129:239–246

Fladby T, Jansen JKS (1988) Selective innervation of neonatal fast and slow fibres before a net loss of synaptic terminals in mouse soleus muscle. Acta Physiol Scand 134:561–562

Gordon H, van Essen DC (1981) Motor units diversify in size as synapse elimination proceeds in the neonatal rabbit soleus muscle. Soc N Sci 7:179

Gouzé JL, Lasry JM, Changeux JP (1983) Selective stabilization of muscle innervation during development: A mathematical model. Biol Cybern 46:207–215

Jansen JKS, Fladby T (1990) The perinatal reorganization of the innervation of skeletal muscle in mammals. Prog Neurobiol 34:39–90

Laskowski MB, Sanes JR (1988) Topographically selective reinnervation of adult mammalian skeletal muscles. J Neurosci 8:3097–3099

Lichtman J (1977) The reorganization of synaptic connections in the rat submandibular ganglion during postnatal development. J Physiol (London) 273:155–177

McArdle JJ (1975) Complex endplate potentials at regenerating neuromuscular junction of the rat. Exp Neurol 49:629–638

O'Brien RAD, Ostberg ASC, Vrbova G (1978) Observations on the elimination of polyneuronal innervation in developing mammalian skeletal muscle. J Physiol (Lond) 282: 571–582.

Redfern PA (1970) Neuromuscular transmission in newborn rats. J Physiol (Lond) 209:701–709

Ribchester RR (1988) Activity-dependent and -independent synaptic interactions during reinnervation of partially denervated rat muscle. J Physiol (Lond) 401:53–75

Ribchester RR, Taxt T (1983) Motor unit size and synaptic competition in rat lumbrical muscles reinnervated by active and inactive motor axons. J Physiol (Lond) 344:89–111

Thompson WJ (1985) Activity and synapse elimination at the neuromuscular junction. Cell Mol Neurobiol 5:167–182

Thompson WJ, Jansen JKS (1977) The extent of sprouting of remaining motor units in partly denervated immature and adult rat soleus muscle. Neuroscience 4:523–535

Malsburg C von der (1973) Self-organization of orientation sensitive cells in the striate cortex. Kybernetik 14:85–100

Willshaw DJ (1981) The establishment and the subsequent elimination of polyneural innervation of developing muscle: theoretical considerations. Proc R Soc London Ser B 212:233–252

Willshaw DJ, Malsburg C von der (1979) A marker induction mechanism for the establishment of ordered neural mappings: Its application to the retinotectal problem. Proc R Soc London Ser B 287:203–234