The Journal of Neuroscience, November 15, 1999, *19*(22):9975–9985

Activity-Driven Synapse Elimination Leads Paradoxically to Domination by Inactive Neurons

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In early postnatal life, multiple motor axons converge at individual neuromuscular junctions. However, during the first few weeks after birth, a competitive mechanism eliminates all the inputs but one. This phenomenon, known as synapse elimination, is thought to result from competition based on interaxonal differences in patterns or levels of activity (for review, see Lichtman, 1995). Surprisingly, experimental data support two opposite views of the role of activity: that active axons have a competitive advantage (Ribchester and Taxt, 1983; Ridge and Betz, 1984; Balice-Gordon and Lichtman, 1994) and that inactive axons have a competitive advantage (Callaway et al., 1987, 1989). To understand this paradox, we have formulated a mathematical model of activity-mediated synapse elimination. We assume that the total amount of transmitter released, rather than the frequency of release, mediates synaptic competition. We further assume that the total synaptic area that a neuron can support is metabolically constrained by its activity level and size. This model resolves the paradox by showing that a competitive advantage of higher frequency axons early in development is overcome at later stages by greater synaptic efficacy of axons firing at a lower rate. This model both provides results consistent with experiments in which activity has been manipulated and an explanation for the origin of the size principle (Henneman, 1985).

Key words: synapse elimination; neuromuscular junction; synaptic competition; Hebb's postulate; synaptic plasticity; model

The circuitry of the nervous system is refined by selection during development (Purves and Lichtman, 1980). The synaptic alterations, including both synapse elaboration and loss, are thought to be mediated by neural activity, but the role of activity is not well understood. One location where structural changes underlying synapse elimination have been directly observed is the neuromuscular junction. In early postnatal life, the innervation at each neuromuscular junction undergoes a transition from contact by several motor axons to contact by a single motor axon. The removal of presynaptic terminals is accompanied by loss of postsynaptic acetylcholine receptors (AChRs) from the muscle fiber membrane at the same sites. Focal blockade of neurotransmission at parts of a junction indicates that activity of AChRs at one site within a neuromuscular junction can cause synapse elimination at inactive sites (Balice-Gordon and Lichtman, 1994). In these experiments, the area that was functional was found to be important: an active motor axon with a large synaptic area could eliminate an inactive synaptic area, but an active motor axon with a small synaptic area was unable to eliminate an inactive region.

Those focal blockade experiments suggest that motor neurons with greater activities have an advantage in eliminating connections from their competitors. This conclusion is consistent with some experiments (Ribchester and Taxt, 1983; Ridge and Betz, 1984). However, it contradicts the interpretation of other experiments (Callaway et al., 1987, 1989), which found alterations in the size of motor units consistent with a competitive advantage for less active motor neurons.

Additionally, in adult muscles, the muscle fibers innervated by each axon (a motor unit) are recruited in an orderly manner according to the size principle (Henneman, 1985). During a muscle contraction, motor units with the smallest size (number of innervated muscle fibers) are always recruited, whereas the largest motor units are only recruited when the greatest muscle tensions are required. Small motor units thus are presumably more active on average than large ones. If developmental activity patterns are similar to adult activity patterns, then axons that are recruited least often during development must have maintained more connections than more active axons, because when competition is complete, relatively inactive motor units are the largest. This inverse relation between activity level and competitive vigor would favor the development of connectivity patterns that are consistent with the size principle but inconsistent with the studies at individual neuromuscular junctions mentioned above. We have attempted to bridge the gap between these two sets of results by asking what the consequence of activity-mediated synaptic competition at individual neuromuscular junctions might be on the size of motor units. We have constructed a model that incorporates activity-driven elimination at individual neuromuscular junctions while also reproducing the inverse relation of the size principle.

Previous work has extensively characterized synapse elimination in the mouse trapezius muscle (Colman et al., 1997). We have used data from this physiological study as our principle test of the accuracy of the model at simulating the actual changes that occur during synapse elimination.

MATERIALS AND METHODS

A detailed explanation and development of the mathematical expressions on which this work is based is found in the Appendix. Briefly, the model

Received July 8, 1999; revised Sept. 2, 1999; accepted Sept. 8, 1999.

This research was supported in part by the National Science Foundation Grant IBN96–34314 (M.J.B.) and by grants from the National Institutes of Health and the Muscular Dystrophy Association (J.W.L.).

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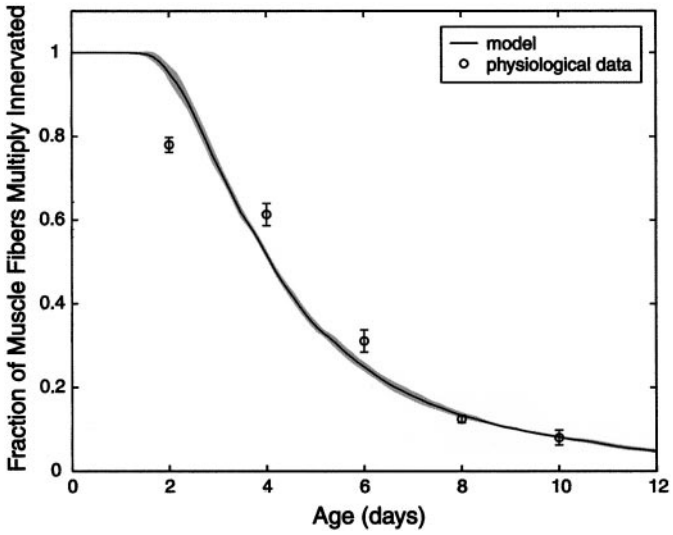

Figure 1. Simulations replicate the experimentally derived time course of synapse elimination. The model (*solid line*) reproduces the experimentally observed time course of elimination (*open circles*). In both cases, an initial period of gradual loss gives way to a period of more rapid loss, which finally tapers off as uniform single innervation is approached. To estimate the variability of the model results, the mean (*solid line*) of the fraction of multiple innervation from 10 simulations with different randomly assigned initial connectivity is presented with the range of variability (SD) shown by the *gray shaded region*. The physiological data shown here (Colman and Lichtman, 1993) were used to determine the rate parameters in the model (data shown are mean ± SEM).

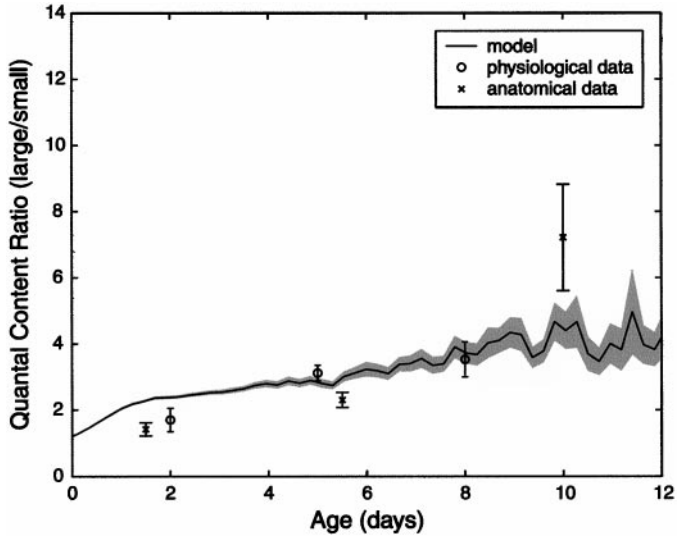

Figure 2. Simulations replicate the increasing disparity seen experimentally between the synaptic areas of axons converging at the same developing neuromuscular junction. The experimentally derived synaptic strength (○; Colman and Lichtman, 1993) and synaptic area (x; Balice-Gordon et al., 1993) maintained by competing axons at a neuromuscular junction are initially similar, but steadily diverge with age. A similar trend is seen in the simulation (*solid line*, *gray shaded region* indicates mean ± SEM). In the simulations, the mean quantal content ratio or area ratio becomes more variable late in the competition period as the number of multiply innervated junctions decreases (experimental data shown are mean ± SEM).

we have developed postulates that two properties of neurons govern changes in synaptic connections during development. First, at every neuromuscular junction, each axon exerts competitive pressure on the other axons innervating the same junction to reduce their synaptic areas. Second, each motor neuron has limited resources (e.g., a limited metabolism), constraining the total synaptic area that it can maintain over the entire motor unit. We have developed a system of coupled, nonlinear differential equations based on these two postulates.

To extract information from the model equations, we resorted to numerical solution. We used a fourth and a fifth order Runge–Kutta method with adaptive time step (Shampine and Allen, 1973), available in Matlab (version 4.2c). This model was computationally tractable. Typical simulations, with several thousand axonal connections, took no more than several hours on a DEC Alphastation (266 MHz; 196 MB).

The number of equations and the manner in which they couple is determined by the connectivity of each axon in the muscle. We determined the initial conditions by specifying the number of muscle fibers, the number of motor neurons, and the initial degree of multiple innervation at each neuromuscular junction (one junction per fiber). We then connected each muscle fiber to a randomly selected subset of motor neurons from the population to produce a specified degree of multiple innervation [see Willshaw (1981) for discussion of randomly assigned connections].

The strength of each synaptic connection (i.e., quantal content or synaptic area of each motor axonal input to each neuromuscular junction) was set at the commencement of each simulation. The initial synaptic strengths varied only slightly (±5%). The metabolic constraint implies that the synaptic area a neuron possesses is either balanced with the capacity of the neuron for support, exceeds the support, tending to cause the neuron to lose area, or under-utilize the capacity of the neuron, allowing the neuron to gain area. We have set the initial areas such that there is a tendency for growth to match the twofold increase in neuromuscular junction area empirically found in early postnatal life (Balice-Gordon et al., 1993).

To accurately model the initial state of a real muscle would require detailed information about all the synaptic areas and patterns of innervation at some early developmental time point. Because this information is not available, the initial conditions were set up to approximate what is known about the state of neuromuscular innervation at birth. This approximation by necessity is only rough and may better correspond to the synaptic connectivity at a slightly different age (prenatal or postnatal). We allow for this possibility by shifting the age at which the simulations commence so as best to match the time course of the elimination of multiple innervation.

Our initial characterizations of the behavior of the model for different values of parameters were performed using small, biologically unrealistic, but rapidly solved, simulations (5–10 motor neurons, 50–200 muscle fibers). We examined the results for multiple sets of initial conditions to insure that our results were reliable and used these initial studies to determine the relevant range of model parameters. We then focused on more realistic and time-consuming simulations (10–50 motor neurons, 500–2000 muscle fibers) for the majority of our studies. In general, the qualitative behavior of both the large and the small simulations were quite similar, although the smaller simulations occasionally showed pathological behavior (e.g., a motor neuron only maintaining an axonal connection to a single neuromuscular junction) not observed in more realistically sized simulations.

RESULTS

Assumptions

The model used in these simulations was developed from four assumptions. First, the ability of an axon to eliminate competing axons at a multiply innervated neuromuscular junction is proportional to the amount of neurotransmitter it releases. Therefore, as a corollary, the eliminative ability of an axon increases in proportion both to its number of active zones (i.e., a measure of synaptic area and quantal content) and to its activity (i.e., mean firing rate). Second, we assume that the total resources of a neuron are limited and constrain the amount of neurotransmitter available for release. Because of this limitation, the amount of neurotransmitter release is adversely affected by large total synaptic areas and high frequency of release. Third, we analogously assume that the limited resources also constrain the total synaptic area the neuron can support. Because of this limitation, the total

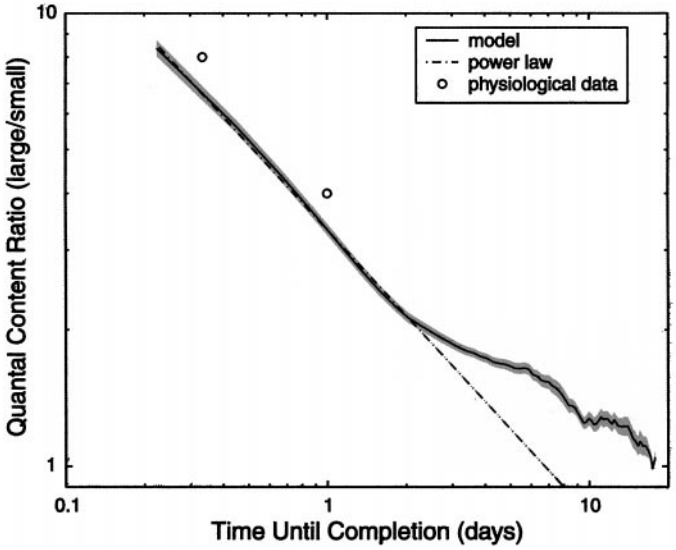

Figure 3. Simulations generalize the experimentally derived relation between the relative strengths of competing inputs and the time required to complete the synapse elimination process. Shown are two experimentally derived estimates (○) of the time remaining until single innervation and the quantal contents of the competing inputs (Colman and Lichtman, 1993). In simulations, the average time it takes the competition to conclude at a doubly innervated junction (*solid line, gray shaded region* indicates mean ± SEM) appears to be related to the quantal content ratio by a power law (*dashed line*). Specifically, for quantal content ratio r and remaining time t, $r = (3.3240)\, t^{-0.6286}$. Note that quantal content ratios approximately <2:1 do not obey this power law, suggesting that the outcome of the competition is still in doubt at neuromuscular junctions in which the difference in quantal contents is minor.

synaptic area is adversely affected by the amount of neurotransmitter released. Fourth, we posit that there is an economy of scale such that larger area synapses are disproportionately less taxing on the resources of the neuron. A possible mechanism for such an economy of scale is provided by the geometry of synaptic terminals: large terminal areas by virtue of their greater volume to surface area ratio use energy more efficiently. West et al. (1997, 1999) have explored the origin of scaling laws of this sort in detail.

From the above assumptions, we have developed a system of coupled nonlinear differential equations (see Appendix). Each equation describes the rate at which the synaptic area of one of the axons changes at a single (singly or multiply innervated) neuromuscular junction. The system of equations thus describes the area changes of every axon at every neuromuscular junction in a muscle. We obtain the time course of the area changes by solving the system of equations. Given the number of equations necessary to model a muscle with a reasonably large number of neuromuscular junctions, we resorted to computer simulations using a standard numerical approach (see Materials and Methods).

The modeled changes in synaptic area will depend not only on the assumptions above, but also on the number, arrangement, and function of the neuromuscular connections. In particular, the innervation patterns of motor units at the commencement of the competition period and the activity levels of the neurons specify the starting conditions of the model. To simulate muscle innervation patterns, we specified a number of starting conditions, including the number of motor neurons (5–50), the number of muscle fibers (50–2000), the synaptic area initially present for each connection (in square micrometers), and the initial degree of polyneuronal innervation of each neuromuscular junction based on known properties. In the simulations presented here, the initial synaptic areas were all set to be approximately equal.

To simulate the behavior of motor neurons, we also needed to set their activity. We varied both the ranges and patterns of activity in accord with work showing that motor axons varied in their overall levels and did not fire synchronously (Henneman, 1985). At one extreme, we tested situations in which the activities of all axons were equal and either synchronous or asynchronous. At the other extreme, we tested highly disparate activity levels such that the most active neuron was many times (~100-fold) more active than the least active neuron. We also controlled how the activity levels were distributed over the population of motor units (in particular, whether the distribution was Gaussian or uniform).

We ran a series of preliminary simulations (using fewer muscle fibers than real muscles; see Materials and Methods) with various starting conditions. We used the results of these simulations to determine a range of conditions that were consistent with typical properties of mammalian neuromuscular systems and that gave results that were consistent with previously obtained anatomical and physiological data. All the simulations of normal development that we will describe here have 1000 muscle fibers innervated by 50 motor neurons. The results described here were based on giving motor neurons uniformly distributed activities ranging from 4- to 20-fold from the most to least active neuron. We found that substantially larger ranges of activities gave qualitatively similar results, as did Gaussian-distributed activities.

Time course of elimination

In a variety of rodent muscles, the transition from uniformly multiple innervation to single innervation is completed during the first several postnatal weeks (Jansen and Fladby, 1990). In each case, experimental studies have shown that this transition occurs in a similar way: a period of gradual elimination is followed by more rapid elimination, which then tapers off to a more gradual rate as uniform single innervation is approached. For example, in mouse trapezius muscle, the transition from multiple to single innervation begins slowly (~3%/d) in the first few days after birth, but the rate increases so that in less than a week it is maximal (~20%/d) and then decreases again until virtually all the fibers are singly innervated at 2 weeks of age (Colman et al., 1997). The simulations we ran approximated this time course, showing the characteristic sigmoidal shape experimentally observed (Fig. 1).

Changes in synaptic strength during synapse elimination

Studies have shown that axon withdrawal at individual neuromuscular junctions is the consequence of a gradual loss of synaptic area and strength by the losing axon (Gan and Lichtman, 1998; Balice-Gordon et al., 1993; Colman et al., 1997). Thus, there is a progressively increasing disparity in quantal content between competing axons, causing a skewing in the ratio of quantal contents (larger input to smaller input). Anatomical studies show a comparable shift in the ratios of the areas occupied by the competing inputs (Balice-Gordon et al., 1993). The simulations we ran matched the skewing of the synaptic strengths or areas observed experimentally (Fig. 2).

An estimate of the length of time necessary to reach single innervation as a function of quantal content ratio was found by comparing, for each day or shorter time period, the ratios of

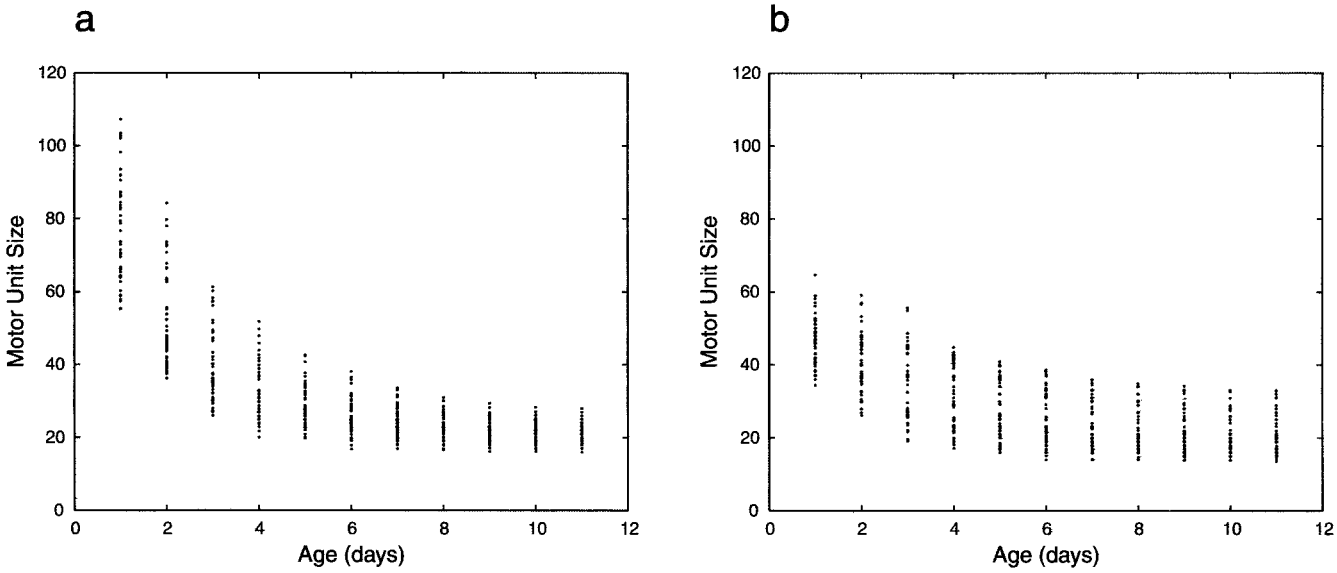

Figure 4. Simulations mimic the trend in motor unit size development that occurs in two different muscles. *a,* Simulations patterned after the soleus muscle (Brown et al., 1976; Jansen and Fladby, 1990), with six axons converging on each neuromuscular junction at birth, show a definite narrowing in the range of sizes with age, whereas simulations patterned after the EDL muscle (Balice-Gordon and Thompson, 1988), in which two or three axons converge (*b*), maintain a similar range of sizes throughout the competition period.

quantal contents of the competing inputs with the number of muscle fibers that became singly innervated on the following day or part of a day (Colman et al., 1997, their Fig. 6). This analysis showed, for example, that once the quantal content ratio (larger axon to smaller axon) of the competing inputs reached fourfold, single innervation occurred within 24 hr (Fig. 3, *open circles*). The simulations were consistent with this fact and indicate a deeper relation between the quantal content ratio and the length of time remaining before single innervation is reached (Fig. 3).

Changes in motor unit size during synapse elimination

The simulation results presented thus far focus on competition at individual neuromuscular junctions, but we also considered changes in the sizes of motor units. Experimental studies in rodent soleus (in which junctions are innervated by approximately six axons at P0) have shown that motor units change in two ways during the period when axons are removing their connections from some muscle fibers (Brown et al., 1976; Jansen and Fladby, 1990). First, all motor units are shrinking in size, and, second, the range of motor unit sizes is narrowing. These two properties were replicated in simulations that started with the same degree of multiple innervation per junction (Fig. 4*a*). We also ran simulations patterned after the data in the extensor digitorum longus (EDL), in which junctions are innervated by fewer axons (~2.5) at birth (Balice-Gordon and Thompson, 1988). Interestingly, in this muscle, the reduction in motor unit size was not accompanied by a narrowing of the range of motor unit sizes, in contrast to results in the soleus. In simulations of the EDL, we also found little change in the range of motor unit sizes with age (Fig. 4*b*). The congruence in the biological and model results suggests that the patterns of innervation at birth account for this difference in range without a need for any additional mechanisms that regulate elimination.

The size principle

In at least some adult muscles, there appears to be a relation between the activities of motor neurons and the sizes of their motor units. In particular, there is a size principle: neurons that get recruited most frequently tend to have motor units that are relatively small, whereas neurons that are activated infrequently tend to have large motor units (Henneman, 1985). This behavior is qualitatively reproduced in our simulations (Fig. 5). This result shows that activity-mediated synapse elimination combined with limited resources allows relatively inactive axons to dominate the competitive milieu.

To summarize, the simulations mimic the normal development and patterns of innervation in mammalian muscle in each way we have tested. We have found no test in which the simulation results are not qualitatively similar to what has been observed. Although we have not systematically optimized the model parameters, small variations in these parameters do not significantly alter the results. We next compared the model results with experimental manipulations of the developmental process.

Experimental manipulation of neural activity

By disrupting normal neural activity patterns, Callaway et al. (1987, 1989) found that alterations in the size of motor units were consistent with a competitive advantage for less active motor neurons. In their experiments, the activity of a subset of the motor neurons innervating a muscle was blocked midway through the competition period. That subset of the neurons that were blocked maintained motor units at the conclusion of the competition period that were slightly larger than normal.

We explored the consequences of manipulating neural activity in a qualitatively similar manner. The initial patterns of innervation and synaptic areas were assigned as described above (with 15 motor neurons and 300 muscle fibers), whereas the neural activities were randomly assigned values between 5 and 20 Hz. These initial conditions were used in two different simulations: first, a simulation of a normal competition, without any manipulation of neural activity; and, second, a simulation with the activity of two of the motor neurons blocked (reduced to 20% of normal) starting partway through the competition process. In this way we could

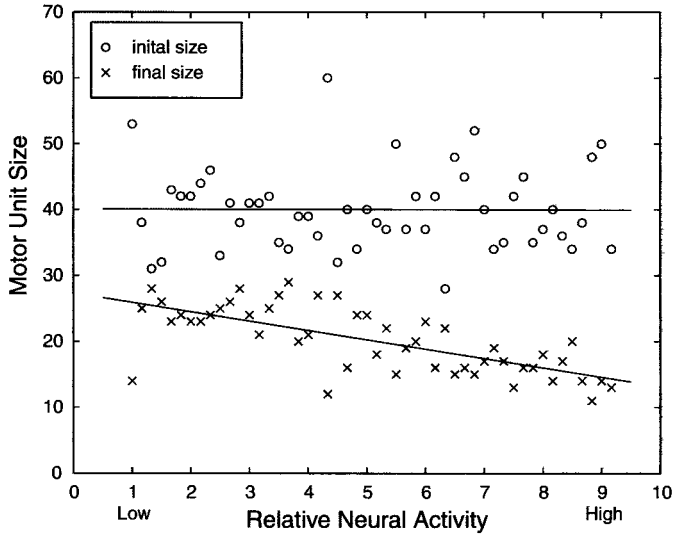

Figure 5. In simulations, the least active motor units maintain the largest sizes. In accord with the experimentally derived size principle (Henneman, 1985), a comparison of the initial sizes (○) and final sizes (x) of motor units shows that the most active axons have a greater decrease in size than the least active axons. The least squares fits to these data sets have significantly different slopes [as a function of activity f, the initial motor unit size is $(-0.0156)f + 40.0791$, whereas the final motor unit size is $(-1.4181)f + 27.3287$].

see whether the degree of remaining multiply innervated muscle fibers affected the outcome of activity blockade. This procedure was followed for multiple sets of initial conditions, showing a small but clear advantage for the blocked motor neurons at maintaining synaptic connections (Fig. 6*a*,*b*).

Experimental manipulation of synaptic connectivity

A variety of pharmacological manipulations alter the time course of synapse elimination (Nguyen and Lichtman, 1996). The most dramatic of these alterations comes in response to abnormally high levels of glial cell line-derived neurotrophic factor (GDNF) (Nguyen et al., 1998). This growth factor causes hyperinnervation of neuromuscular junctions by as many as eight different motor axons, whereas control muscles typically have only two motor axons converging on each neuromuscular junction during early postnatal life. Besides the additional innervation, GDNF-treated muscles reach a state of single innervation several weeks later than control muscles. It is not clear whether the delay is a consequence of the longer time it may take to eliminate extra axons or whether the GDNF has a deleterious effect on the efficiency of synaptic competition (or perhaps whether both are occurring). To attempt to resolve this issue, we simulated the effect of GDNF on the degree of axonal branching.

In each of our GDNF simulations, we set all the muscle fibers to be hyper-multiply innervated with a variety of initial distributions of the degree of innervation. The total synaptic area at each neuromuscular junction was assigned the same value as used in normal muscle simulations. Thus, each motor axon begins with more synaptic terminals than normal, but each of these terminals occupies a smaller area than normal. From these modified initial conditions, we then ran simulations with the same model that we applied to the normal muscle.

We examined the results of each simulation in two ways. First, we tested the results to see if they were consistent with an altered initial pattern of innervation, but with no alteration in the efficiency of synaptic competition. For this analysis, we adjusted the starting age so that the simulation results best matched the experimentally observed fraction of muscle fibers that were multiply innervated. We then used the time course determined in this

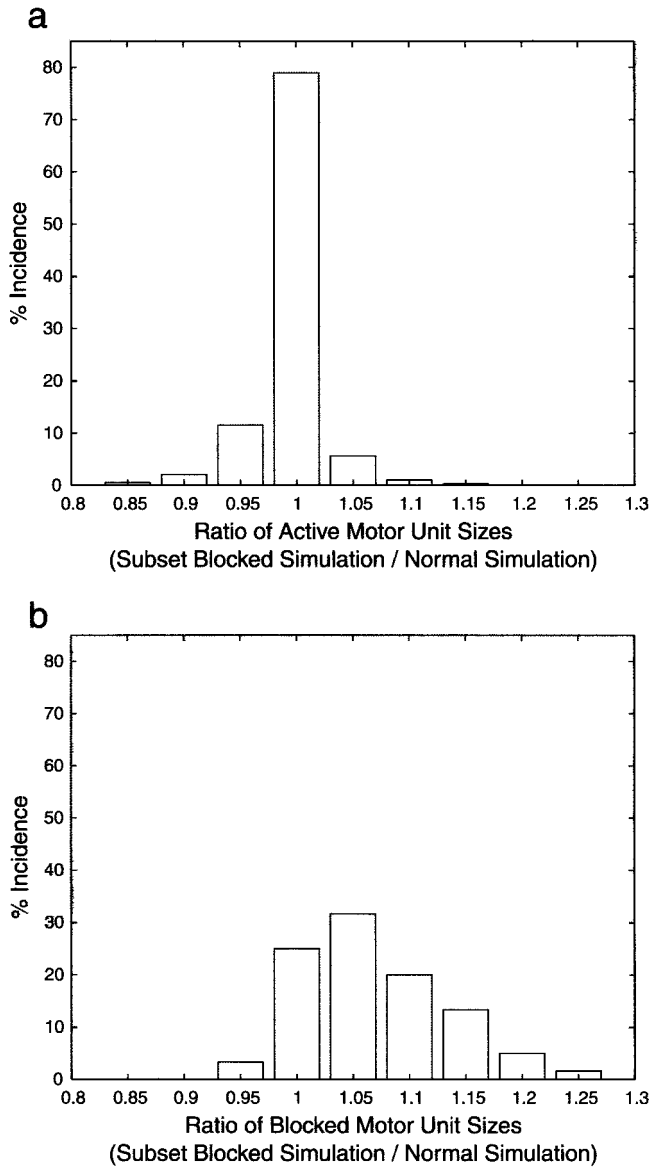

Figure 6. In the simulations, blocking neural activity in a subset of motor axons increases the ability of the blocked axons to maintain synaptic connections. For each of thirty sets of initial conditions, two simulations were run: a normal simulation and a simulation in which a subset of 2 of 15 motor neurons were blocked for the latter half of the competitive period (days 5–12). The ratios of the sizes of the same motor units in the two simulations (subset blocked and normal) were calculated. The results were segregated into the effects on motor units that were active in both simulations and motor units that were blocked in one of the simulations. *a,* This histogram shows the ratios of motor units whose activities were normal in both the subset blocked and normal simulations. The ratios calculated for these motor units are nearly symmetrically distributed around ~1. *b,* This histogram shows ratios for the minority of motor units whose activities were blocked in subset blocked simulation. For these motor units there is a rightward shift in the histogram, indicating that blocking activity increased their ability to maintain connections, consistent with the experimental findings of Callaway et al. (1989). The differences between the distributions in a and b are highly significant ($p < 0.0001$; two-sided Mann–Whitney U test).

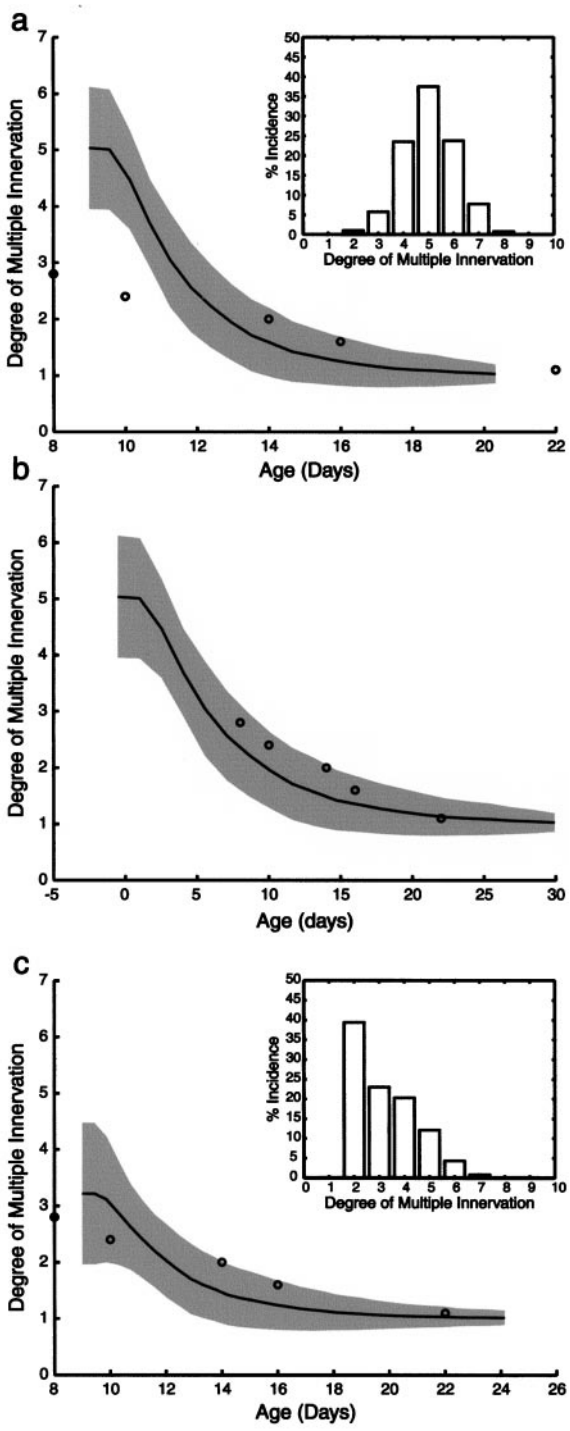

Figure 7. Tests of possible alternatives for the cause of the maintained hyperinnervation associated with GDNF overexpression (Nguyen et al., 1998). *a,* For a GDNF-treated muscle in which we simulated a Gaussian-distributed initial degree of hyper-multiple innervation (*inset*), the model produces a time course of synapse elimination (*solid line*) and SD (*gray shaded regions*) that is inconsistent with the experimental evidence (○). The differences between the model and experimental results are significant ($p < 0.05$; χ^2 test). *b,* However, when we also change the rate of competition by adjusting the model parameters, the simulation results are consistent (within one SD from the mean) with the experiments. The differences between the model and experimental results are not significant. *c*, Conversely, for strongly skewed initial distribution of axonal convergence (*inset*), simply changing the initial distribution of axonal convergence produces results in accord with the experiments. The differences between the model and experimental results are not significant. Thus, the model suggests that GDNF could generate its effect on synapse elimination in two different ways.

way to examine the development of the degree of multiple innervation in the simulations. Second, we altered the rate of competition as well as the starting age in the simulations to best match the experimentally observed fraction of muscle fibers that were multiply innervated. We again used this optimal time course to examine the development of the degree of multiple innervation in the simulations.

In a simulated muscle in which the initial degrees of hyper-multiple innervation were Gaussian-distributed, the delay in the attainment of single innervation was not explained by only a change in the initial pattern of innervation (Fig. 7*a*). Altering the rate of competition did yield results consistent with the experiments (Fig. 7*b*), suggesting that GDNF has a deleterious effect on synaptic competition. However, in simulations in which the initial degrees of innervation were distributed in a strongly skewed fashion, the opposite result was found: results consistent with the experiments were obtained by altering the initial patterns of innervation, without altering the rate of competition (Fig. 7*c*). These results suggest that GDNF may not act directly to alter the efficiency of synaptic competition, but rather induces branching of axons and thus affects the starting point of the competition.

The initial distribution of the degrees of multiple innervation before birth in real muscles is not yet known. Thus, we are not able to draw a definite conclusion about the effect of GDNF on the efficiency of synaptic competition. Our results suggest that careful analysis of the incidence of single, double, and other degrees of multiple innervation should be sufficient to clarify this issue.

DISCUSSION

Although several formal models for the elimination of multiple innervation have been constructed, we were motivated to generate another model because most of the present models do not address the role of activity or link the effects of activity at individual neuromuscular junctions with its ultimate effect on the size of motor units. Previous models have focused more on deducing the mechanisms by which connections are maintained and removed rather than understanding the role of activity in the elimination of connections.

Several models have based elimination on competition for trophic factors in limited supply. The earliest of these, by Gouzé et al. (1983), considered competition for a postsynaptic resource, with small random differences in the initial amount of trophic factor becoming magnified through a competition process until only a single terminal remained. Another model, proposed by Bennett and Robinson (1989) and extended and clarified by Rasmussen and Willshaw (1993), combined competition for both presynaptic and postsynaptic resources, with both resources necessary for synaptic adhesion. van Ooyen and Willshaw (1999) further analyzed this model, showing that it could also explain the persistent multiple innervation at neuromuscular junctions that underwent a period of prolonged inactivity caused by chronic nerve conduction blockade. A third model, by Jeanprêtre et al. (1996), used a detailed consideration of competition for postsynaptic trophic factors at a single target cell. Eliott and Shadbolt (1996, 1998) also modeled competition for postsynaptic trophic factors at a target cell, assuming it to be driven by activity, but do not analyze the large-scale effects of activity or competition across the entire set of target cells innervated by an axon.

Phenomenological models have also been proposed; these models assume mechanisms for the competitive elimination of axons that do not involve trophic factors in limited supply.

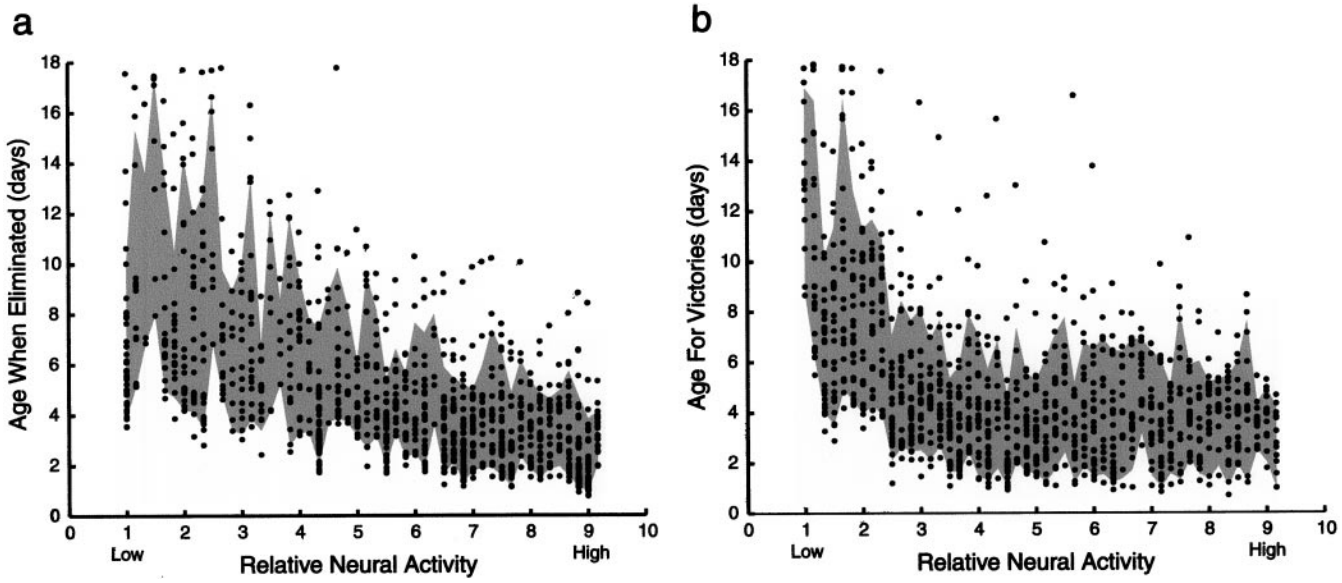

Figure 8. Simulations predict that the time ranges over which a motor unit is contracting and over which it is eliminating competitors is related to its activity level. *a,* Relatively active neurons tend to contract their motor units early in the competition, whereas relatively inactive neurons tend to contract their motor units late in the competition. The simulation has 50 motor neurons, each of which has a distinct activity level. The *dots* in each *vertical line* represent the times at which the inputs of a motor axon are removed from each neuromuscular junction. The *shaded gray regions* (*a*, *b*) include the middle 80% of the observations to trim outliers. *b,* Relatively active neurons tend to eliminate competing axons early in the competition, whereas relatively inactive neurons tend to eliminate competing axons late in the competition. The *dots* in each *vertical line* represent the times that a motor axon with a particular activity level eliminates the competing axon at each neuromuscular junction. In both *a* and *b*, it can be seen that, for each axon, the majority of changes in connectivity it undergoes and causes in competitors occur over only a few days.

Willshaw (1981), for example, assumed that each motor axon injects a degrading signal into its endplate region that reduces the "survival strength" of the terminals at that endplate; this model incorporates a presynaptic resource constraint (as does the present model) so that the total survival strength of the terminals of each neuron is maintained at a fixed level. This model successfully reproduced synapse elimination but did not incorporate activity in an explicit fashion. Smalheiser and Crain (1984) also discuss a "sibling neurite bias" idea in which presynaptic constraints influence the synaptic competitions at the neuromuscular junction. Although this hypothesis (not fully developed as a formal mathematical model) considers presynaptic and postsynaptic constraints, it does not include a postsynaptic role for activity in synapse elimination and thus does not provide a framework for analyzing the central paradox of the role of activity. Van Essen et al. (1989) considered a number of possibilities, but examined in greatest depth the possibility that terminal growth depends on how much "scaffold" is incorporated into the underlying basal lamina. Their models allowed a competitive advantage for inactive axons but did not provide an explanation for the activity dependence of synapse elimination later found (Balice-Gordon and Lichtman, 1994). Stollberg (1995) considered "correlational competition" learning rules that led to the establishment of the size principle. This broad class of rules is appropriate for the kind of analysis we undertook. In fact, the model we propose can be viewed as being driven by correlations in presynaptic and postsynaptic activity. A major difference is that the present work is based more on particular experimental results, whereas the correlational competition approach is based more on a theoretical analysis of Hebbian rules.

In this work we have taken a phenomenological approach to modeling synapse elimination. Rather than hypothesizing that, for example, nerve activity induces uptake of a putative trophic factor, we have attempted to incorporate several conventionally accepted facts about activity. Whereas this model therefore is incapable of describing the fundamental mechanisms, it has the advantage that it directly describes the phenomenon at the level at which questions raised by the model can be answered experimentally. This property of the model provides us with interesting and testable predictions about the properties of axons involved in synaptic competition.

One prediction is that, in addition to the expected relation between the activity level of a neuron and the size of its motor unit, a relation exists between the activity level and the timing of synapse elimination. In particular, early in the competitive period, the connections of a relatively active neuron are withdrawn, whereas the connections of a relatively inactive neuron are withdrawn later (Fig. 8*a*). In addition, this relation indicates that the majority of the change in the motor unit size of a neuron should occur over only a few days, rather than spread across the entire competition period.

A second prediction is that a relation exists between the activity level of a neuron and the ages at which its axonal connections win the competitions at individual neuromuscular junctions. Thus, axons of relatively active neurons are victorious early in the competitive period, whereas axons of relatively inactive neurons win later (Fig. 8*b*). Taken together, these two predicted trends suggest that early competition is dominated by active axons pitted against other active axons, whereas the main changes in connectivity are dominated later by relatively inactive axons that battle other inactive axons. Given this framework, experimental examination of the firing rates of different axons during early postnatal life should provide useful data for understanding the underlying mechanisms that regulate motor unit size and synaptic competition.

The model also provides a description of how the synaptic areas change at each neuromuscular junction throughout the elimination period (Fig. 9). A number of additional unanticipated

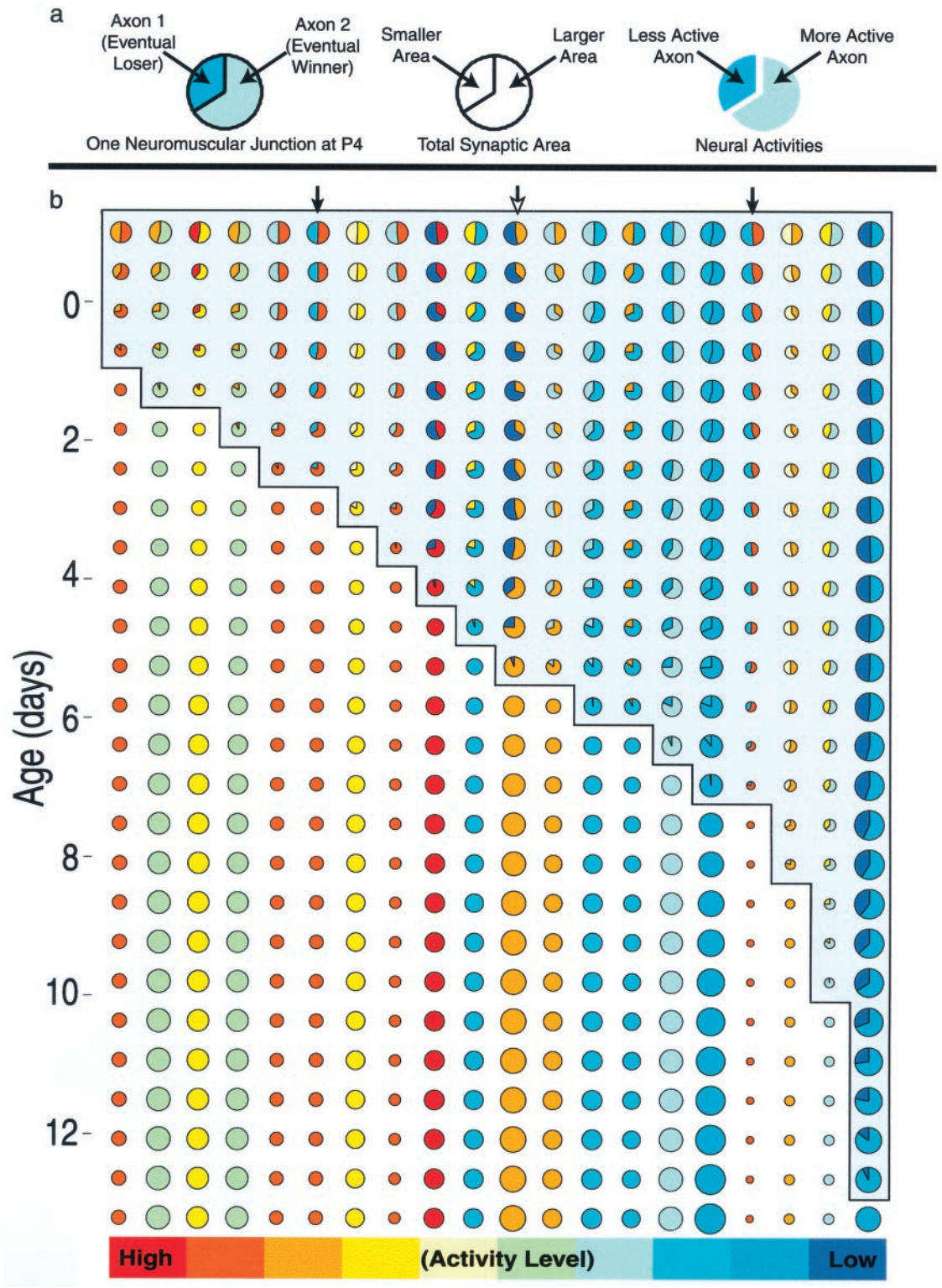

Figure 9. The time development of individual simulated neuromuscular junctions shows many variations on a general trend. *a, Left*, Each neuromuscular junction is depicted as a *color-coded circle*, with both the synaptic areas and the axonal activities simultaneously presented. *a, Center*, For each doubly innervated neuromuscular junction, the area of the *circle* represents the total synaptic area at the neuromuscular junction. The *circle* is divided into two wedges with areas proportional to the areas maintained by each of the innervating axons. *a, Right*, The axonal activities are shown by the color, with the most active axons shown in *red* and the least active in *dark blue* (all neuromuscular junctions innervated by a particular axon are shown in the same color). *b,* Here, we show the changes that occur for 20 neuromuscular junctions, taken from a simulation consisting of 10 motor neurons and 200 muscle fibers, over a period of 12 d (14 hr between time points). The data are presented in such a way that the area of the ultimately victorious axon (*wedge*) at each neuromuscular junction (*circle*) gains by moving in a clockwise direction.

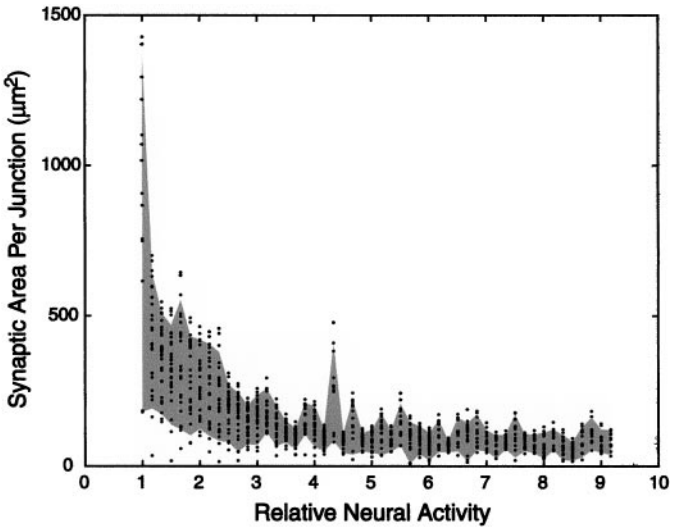

Figure 10. The model predicts that, after synapse elimination is complete, neuromuscular junction area is inversely related to the activity level of the innervating axon. The *dots* in each *vertical line* show the synaptic areas maintained at individual neuromuscular junctions by a motor axon with a particular activity level. The *gray shaded region* shows the range of the middle 80% of the data to trim outliers.

trends are seen. First, at each neuromuscular junction changes in the synaptic areas are not spread uniformly over the competition period. Rather, after a period of relative quiescence of varying length, there is a period of several days during which the endplate undergoes rapid change in area. Thus, neuromuscular junctions that complete synapse elimination early (Fig. 9, *left*) have a negligible quiescent period, whereas those that complete the process later begin with a lengthy quiescent period (Fig. 9, *right*). Notably, the significant changes in synaptic structure occur over a similar length of time for all junctions.

Second, despite already noted trends, it is impossible to predict the duration of multiple innervation at a particular junction based solely on the activity levels of the competing axons. For example, the filled arrows (Fig. 9) indicate two neuromuscular junctions at which the same competing axons take substantially different lengths of time to resolve the competition. The integrated effects of synaptic competition going on simultaneously at many different sites have amplified small differences in initial synaptic areas.

Third, at some neuromuscular junctions, the competition is impacted by this collective effect so strongly that the areas do not proceed monotonically toward the end state. In particular, the open arrow (Fig. 9) shows a neuromuscular junction at which there is an early trend in favor of the purple axon. However, this trend reverses, and the orange axon is ultimately victorious.

Fourth, it is also clear that the relative activity of the competing inputs to a muscle fiber does not determine unambiguously who will ultimately be eliminated. Despite the overall trend favoring the elimination of relatively active axons, there are many instances in which relatively active axons instead eliminate the competing axons (Fig. 9).

Fifth, it is also apparent that the ultimate size of a junction is related to the activity of the axon that is maintained. In particular, the area is inversely related to the activity of the axon (Fig. 10). Because neuromuscular junctions do range in area, it will be interesting to examine whether this variability is indeed secondary to activity levels.

Conclusions

Our main goal has been to take two sets of paradoxical results and incorporate them into a single model. Our attempt to do this has been largely successful and has provided a possible resolution for the paradox. In particular, we have reproduced the observed trends that (1) active neurons maintain small motor units, but (2) activity drives competition at the neuromuscular junction. This consolidation was accomplished by considering the global redistribution of synaptic resources as local competition eliminated axonal connections at individual neuromuscular junctions. By considering the dynamic global environment in which local competition is taking place, we have gained a better understanding of the relationship between global outcomes and the local phenomena that drive them.

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APPENDIX

To undertake the study discussed in this work, we formulated a model system of differential equations based on focal blockade experimental studies of synaptic elimination (Balice-Gordon and Lichtman, 1994) and a consideration of the costs a neuron must pay to maintain a given level of activity. We assume that neurons have finite resources that constrain the availability of neurotransmitter and the total synaptic area the neuron can maintain. To relate our theoretical findings to a variety of experimental findings, we assume that the quantal content of an axonal connection at a neuromuscular junction is directly proportional to the synaptic area that the axon maintains at that junction.

Each synaptic connection will thus be subjected to two effects: (1) elimination of synaptic area through activity-mediated competition and (2) gain or loss of quantal content (and, thus, synaptic area) through activity-mediated utilization of neuronal resources. The change in synaptic area that results from these effects is:

$$\frac{dA_{mn}}{dt} = -\alpha E_{mn} + \beta U_{mn}, \tag{1}$$

where A_{mn} is the synaptic area that neuron n makes on muscle fiber m, E_{mn} is the synaptic area lost because of competition, and U_{mn} is the gain or loss of area on muscle fiber m as a consequence of the limited resources of neuron n. The relative importance of these two effects is determined by the rate constants α and β. There will be an equation of this sort for each connection on each muscle fiber. There are N neurons and M muscle fibers.

We deduce the form of the elimination term E_{mn} from the focal blockade experiments (Balice-Gordon and Lichtman, 1994). As such, large synaptic areas have a greater ability to eliminate competitors than small ones. Similarly, more active axons have a greater ability to eliminate competitors than less active ones. The outcome of the competition occurring at one muscle fiber has no direct effect on the competition occurring at other fibers (although the resource constraint causes profound indirect effects in our model).

We incorporate these experimental findings by first considering the competition between two axons (corresponding to neurons n and i) at the neuromuscular junction of muscle fiber m. We define E_{mni} as the loss of synaptic area by axon n caused by competition with axon i, and take:

$$E_{mni} \propto f_i A_{mi}, \tag{2}$$

which increases both with the synaptic area A_{mi} and the average activity f_i of neuron i.

Because only asynchronous activity is important, we must define E_{mni} to prevent the loss of synaptic area A_{mn} during periods of synchronous activity. The precision of the timing needed to consider the activity of the axons to be synchronous is not known, nor is it clear what constitutes an appropriate measure of activity. The focal blockade experiments avoided this issue entirely and thus provide no helpful information. We interpret activity as the mean firing rate of the neuron; this activity is assumed to remain constant over the competition period, except where otherwise noted.

Despite the uncertainties in activity, we can account for synchronous activity in at least an approximate way. If neuron n maintains an activity rate f_n for some time, then the fraction of that time in which the neuron is active may be expressed as τf_n (based on dimensional considerations, the proportionality constant τ has units of time; it may be viewed as defining a time "window" for synchronous activity). If we make the assumption that the activities of the competing neurons are uncorrelated, two different neurons n and i are thus synchronously active a fraction $(\tau^2 f_n f_i)$ of the time and are only able to effectively compete during a fraction $(1 - \tau^2 f_n f_i)$ of the time. This assumption is not experimentally justified; it provides a simple means to estimate the magnitude of the effect of interaxonal synchrony in activity patterns but also limits the range of applicability of this model. Interaxonal correlations in activity had little effect (at most, a few percent) in all simulations presented in this work but could conceivably be very significant in simulations based on experiments in other muscles. However, in these suppositional muscles, any interaxonal correlations in activity would also be quite significant, so synchronous activity would necessarily have to be accounted for in more detail than in the present work.

A final issue that must be considered is the manner in which more than two axons compete. A greater synaptic area contacted by a particular neuron implies a greater ability to eliminate competing axons. The ability of the individual release sites of a neuron to eliminate competing axons thus increases as the number of release sites increases, suggesting an additive process for combining the elimination signals from each release site. Thus, we assume that the elimination signals from release sites of multiple axons are deleterious to a different axon in an additive way. So,

$$E_{mn} = \sum_{i \neq n} E_{mni} = \sum_{i \neq n} f_i A_{mi}(1 - \tau^2 f_n f_i) \tag{3}$$

represents the total effect of competitive elimination acting on the synapses of neuron i at the neuromuscular junction of muscle fiber m. Thus, for the case of three neurons competing at a single neuromuscular junction, when two axons are active (separately or synchronously), the third axon is subjected to competitive pressure. The total area of activated synapses, rather than the particular axons activating those synapses, is what is important.

The elimination term acts only to reduce the area of each synaptic connection. When the synaptic area decreases below some small threshold amount, $A_{\min}$, the connection is eliminated.

The second effect, activity-mediated utilization of neuronal resources, acts on all the terminals of a neuron throughout the entire muscle, rather than influencing an input only at a single neuromuscular junction. We assume that each neuron has limited resources used to make neurotransmitter available and to maintain or enlarge its total synaptic area. Neurotransmitters are distributed among all the synaptic connections of the neuron and are depleted by use (i.e., activity), with a larger total synaptic area having a greater depletion with each impulse. Thus, large and active axons use large amounts of neurotransmitter and neuronal resources, whereas smaller and less active axons use a smaller amount.

This suggests that the cost to maintain a given level of activity should be the product of activity and of a function $g(A_{1n}, A_{2n}, \ldots, A_{Mn})$ of the synaptic areas that neuron n makes on the M muscle fibers. We do not expect the total cost of maintaining a given level of activity to decrease as the total synaptic area increases, so we will require $g(\,)$ to be an increasing function (although an economy of scale may exist, see below, that would change the unit cost for maintaining activity). An immediate consequence of this form for the resource depletion term is that those synaptic connections that are large and active (and therefore least likely to be eliminated) should also be the synaptic connections that most restrict the growth of other connections of the same neuron.

We incorporate this second effect, gain or loss of synaptic area through activity-mediated competition, as:

$$U_{mn} = \left(R_n - f_n \sum_j A_{jn}^{\gamma} \right) \frac{A_{mn}}{\sum_j A_{jn}}. \quad (4)$$

In this paper, we assume that all neurons have the same resources available, $R_n = R$. We have taken the increasing function of the synaptic area to simply be the sum of the A_{mn} to a power γ. This allows for the possibility that neurons with different distributions of synaptic area may use resources more or less efficiently. Taking $\gamma < 1$ represents an economy of scale, where larger synapses produce neurotransmitter more efficiently than smaller synapses; taking $\gamma > 1$ represents a diseconomy of scale, with the opposite effect. The resources are divided among all of the connections of the neuron, with the largest synaptic contacts receiving a greater share of the resources. The resource utilization term may lead to either increasing or decreasing synaptic area in this model.

Substituting our representations of these two effects (competitive elimination and resource utilization) back into our original equation gives:

$$\frac{dA_{mn}}{dt} = -\alpha \sum_{i \neq n} f_i A_{mi}(1 - \tau^2 f_n f_i) + \beta \frac{A_{mn}}{\sum_j A_{jn}} \left(R - \sum_j f_n A_{jn}^{\gamma} \right). \quad (5)$$

As previously noted, there must be one equation of this form for each synaptic connection. When connections are eliminated, the corresponding equation must also be eliminated from the system of model equations.

Main parameter values used for the simulations presented in this work are $\alpha = 0.0798$ sec/d, $\beta = 0.7293\ \mu m^{1/2}$ sec/d, $\gamma = 0.75$, $A_{\min} = 12\ \mu m^2$, $\tau = 1.82$ msec, and $R = 5159\ \mu m^{3/2}$ sec. In those simulations in which we modified the rates, α and β were kept in the same proportion. The value of γ corresponds to an economy of scale in all simulations presented herein.