

Activity-Dependent Synaptic Plasticity: Insights from Neuromuscular Junctions

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Experience-dependent editing shapes synaptic connections throughout the developing nervous system, but the underlying cellular mechanisms remain poorly understood. A useful model synapse for addressing these mechanisms is the neuromuscular junction, the connection between spinal motor neurons and skeletal muscle fibers. Here the authors review current ideas about the role of activity in editing neuromuscular synaptic connections. A variety of new tools are being used to address some unanswered questions in vivo and in vitro. Understanding activity-dependent plasticity at developing neuromuscular synapses may reveal how neural circuits in the central nervous system are altered by experience throughout life. *NEUROSCIENTIST* 8(5):414–422, 2002. DOI: 10.1177/107385802236970

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The precise patterning of synaptic connections, essential for nervous system function, is shaped during neural development by patterns of neural activity that emerge as neurons become connected to their targets. Throughout the central and peripheral nervous system, a neuron's synapses with some targets are strengthened and maintained, whereas its synapses with other targets are gradually weakened and are ultimately deleted. This process, often called "synapse elimination," takes place during late embryonic and postnatal life over a period of several weeks to months. Synapse elimination is a somewhat inaccurate descriptor of this process, however, because both synaptic size and the total amount of pre- and post-synaptic machinery increase dramatically at the same time that some inputs and their synapses are lost. A more accurate term may be "synaptic editing": decisions about synapse retention and elimination edit the pattern of neuronal connectivity and result in the gradual emergence of neural circuits that are fine-tuned to subserve a wide variety of behaviors.

Neural activity modulates the strength and structure of synapses of different inputs that converge on the same target cell in a more or less Hebbian fashion. Over the last two decades, stimulated by work from Hubel, Wiesel, and colleagues on the development of visual

system circuitry (cf. LeVay and others 1980), many studies have suggested that inputs that fire coordinately with postsynaptic cells are generally strengthened and structurally reinforced (reviewed in Katz and Shatz 1996). On the other hand, inputs that fire incoordinately with active postsynaptic cells are generally weakened and eliminated. Activity-dependent and -independent signals exchanged by synaptic partners affect a wide repertoire of neuronal functions, including synaptic maintenance. Relatively little is known, however, about how neural activity modulates this anterograde and retrograde signaling at the cellular or molecular level. This is due, in part, to the relative inaccessibility of CNS synapses and to the dynamics of synaptic plasticity, which require fine spatial and temporal resolution to unravel mechanistically.

Vertebrate neuromuscular junctions, consisting of presynaptic motor axons and terminals, postsynaptic muscle fibers, and perisynaptic Schwann cells (Fig. 1), have proven to be useful model synapses to address these issues. During embryonic and early postnatal life, most vertebrate muscle fibers are innervated by several motor neurons (Fig. 2), but adult muscle fibers are typically innervated by a single motor neuron (Redfern 1970). The transition from multiple to single innervation occurs in most muscles during the first weeks after birth. Multiple innervation is the result of the convergent, suprathreshold innervation of individual muscle fibers by several motor axons (Brown and others 1976). The loss of multiple innervation occurs well after the period of motor neuron cell death and is due to the retraction of some of the axon branches of each motor neuron without a change in motor neuron number (Brown and others 1976; Balice-Gordon and Thompson 1988). Each motor unit, consisting of a motor neuron and all of the muscle fibers it innervates, is thus reduced in size as a consequence of synapse elimination. Many features of devel-

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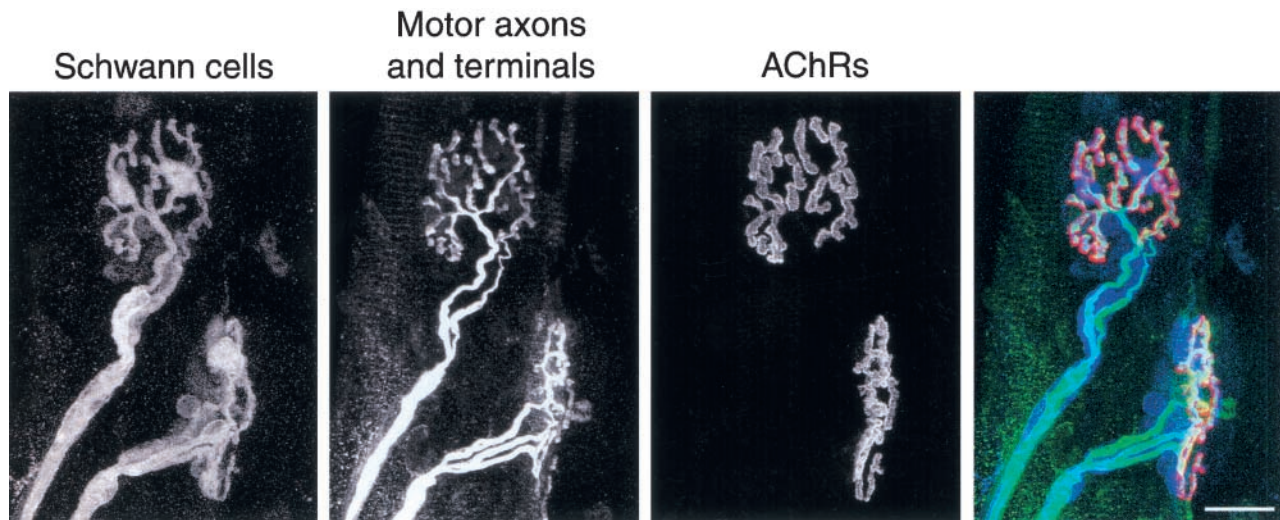


Fig. 1. Cellular components of adult neuromuscular synapses. Immunostaining and confocal microscopic analysis of two neuromuscular junctions from the sternomastoid muscle of young adult mice, with antibodies against perisynaptic Schwann cells (*left*) or motor axons and nerve terminals (*left middle*), and fluorescently conjugated α -bungarotoxin to label postsynaptic acetylcholine receptors (AChRs) in the muscle fiber membrane (*right middle*). An overlay of these three cell types (*right*) shows the precise alignment between pre- and postsynaptic specializations (Schwann cells in blue, motor axons and terminals in green, AChRs in red) and the innervation of each junction by a single motor axon branch, which are hallmarks of adult neuromuscular synapses. Note that the preterminal axons branch once or twice near each of these junctions. Each panel is a single plane projection of a confocal stack of 16 images (Leica TCS 4D system). Scale bar = 20 microns.

opmental synapse elimination are recapitulated after axon injury in adults. Transiently denervated muscle fibers become multiply re-innervated, followed by a period of synapse elimination that continues until muscle fibers are again singly innervated (Rich and Lichtman 1989; Barry and Ribchester 1995). The stable pattern of single innervation of each muscle fiber is essential for the orderly recruitment of motor units during force generation and for normal motor function.

Studies of muscle fibers, as well as of peripheral and central neurons, suggest that target cells are rarely if ever denervated, even transiently, as a consequence of synapse elimination. This suggests that the process of branch withdrawal and synapse loss is not random. Seminal studies by Thompson, Jansen, and colleagues showed that when some motor axons are removed from muscles by cutting them, resulting in partial denervation, the remaining motor neurons retained innervation with more target cells than normal (Thompson 1977; Fladby and Jansen 1987). These observations have been used to argue that inputs actively compete with one another; when the number of competitors is reduced, each input innervates more target cells. This and related work contribute to the view that different inputs actively compete with one another to maintain innervation of the same target cell. Recent work has focused on understanding how synaptic competition results in the loss of some inputs and the maintenance of others at neuromuscular and other synapses.

Cascade of Structural and Functional Changes during Synapse Elimination

Over the last several years, studies in rodents *in vivo* as well as *in vitro* have uncovered some of the cellular

events that occur during synaptic competition during the first weeks after birth (Fig. 3). During this time, changes in the structure and strength of each input's synapses occur in parallel. As multiple innervation of muscle fibers is established, each input has relatively equal presynaptic terminal area (Balice-Gordon and others 1993; Gan and Lichtman 1998), occupies relatively equal areas of postsynaptic acetylcholine receptor (AChR) rich membrane (Balice-Gordon and Lichtman 1993; Gan and Lichtman 1998), and has relatively equal synaptic strength as measured by quantal content (Colman and others 1997). Thus, early in development, there appears to be a relative balance of power among competing inputs, only one of which eventually will be maintained at each neuromuscular junction.

Over time, one input gradually gains as other inputs lose synaptic territory. Using transgenic mice expressing spectral variants of green fluorescent protein (GFP) in only one or two motor neurons per muscle (Feng and others 2000), Keller-Peck and others (2001) recently showed how individual motor neuron branches are pruned during synapse elimination. The use of these transgenic mice allowed synapse elimination to be observed as it played out across the axon branches and terminals of single motor units. This work confirmed the results of other studies demonstrating that the terminals of motor neurons convergently innervating the same muscle fiber are initially extensively intermingled (Balice-Gordon and others 1993) but gradually become segregated into nonoverlapping synaptic regions (Gan and Lichtman 1998). The terminals of each of the labeled motor neuron's axon branches were observed to be at different stages in the process of synapse elimination, providing direct support for the idea, surmised from

Motor axons, terminals AChRs

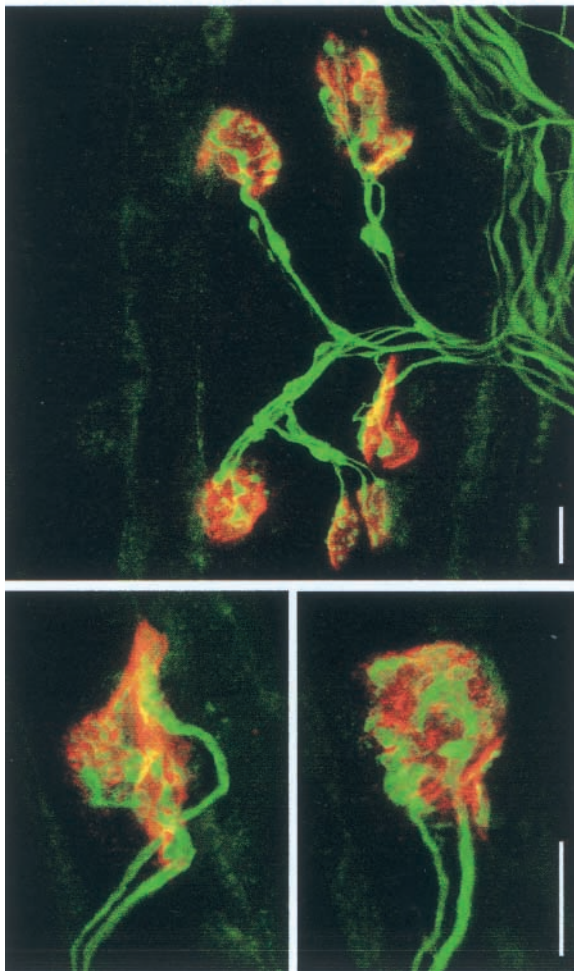


Fig. 2. Multiple innervation of neuromuscular junctions in perinatal mice. Immunostaining and confocal microscopic analysis of neuromuscular junctions from the soleus muscle of P7 mice, with antibodies against motor axons and nerve terminals (green), and fluorescently conjugated α -bungarotoxin to label postsynaptic acetylcholine receptors (AChRs) (red) in the muscle fiber membrane. *Top*, By the end of the first week after birth in this leg muscle, branches of at least two different motor axons can be seen innervating most neuromuscular junctions. From Kopp and others (2000). *Bottom*, Higher magnification view of two dually innervated neuromuscular junctions, with each axon occupying postsynaptic AChR regions. From Personius and Balice-Gordon (2001). Each panel is a single plane projection of a confocal stack of 8 to 10 images (Leica TCS 4D system). Scale bars = 20 microns.

previous work, that elimination occurs at different times across a motor unit. The work of Keller-Peck and others also provides strong support for the idea that synapse elimination is mediated by local competitive interactions that play out at different times within each neuromuscular junction.

As competition begins, a progressive strengthening of some inputs and weakening of others is evident both functionally and structurally within individual neuromuscular synapses. Quantal content becomes increasingly disparate among competing inputs (Colman and others 1997), and the density of AChRs beneath some

inputs is sharply reduced (Balice-Gordon and Lichtman 1993), probably contributing to an observed reduction in their quantal efficacy (Colman and others 1997). The progressive loss of presynaptic terminals (Balice-Gordon and others 1993; Gan and Lichtman 1998) decreases the effective area for weakened inputs to release neurotransmitter. Although a reduction both in postsynaptic AChR density and in presynaptic terminal area contribute to the weakening of inputs, neurotransmitter release probability also affects synaptic strength. Analysis of quantal content and paired pulse facilitation showed that neurotransmitter release probability differs dramatically among competing inputs, contributing to the increasing disparity in quantal content among competing inputs (Kopp and others 2000). These results suggested that inputs with low neurotransmitter release may be at a competitive disadvantage for synaptic maintenance. Recently, preliminary work from Lichtman and colleagues using mice expressing two different spectral variants of GFP in small numbers of motor neurons innervating the same muscles suggested that losing axons can, at least in some cases, withdraw from junctions without loss of postsynaptic AChR regions, and that winning axons can capture this vacated territory (Walsh and Lichtman 2001). Although this suggests that inputs may compete for capture of the same synaptic sites, how this is related to the frank loss of postsynaptic AChRs by losing axons observed in development (Balice-Gordon and Lichtman 1993), in partially blocked adult junctions (Balice-Gordon and Lichtman 1994), and during reinnervation (Rich and Lichtman 1989) remains to be determined. Nonetheless, an increase in postsynaptic area occupied by winning axons would be predicted to strengthen those inputs, contributing to the disparity in synaptic strength between winning and losing axons.

Taken together, this work suggests that synaptic loss and possibly also the capture of synaptic territory may contribute to an increasing disparity in synaptic territory and thus the synaptic strength of competitors. Cycles of functional weakening and structural loss continue until all of the sites innervated by weakened inputs are eliminated. Synapse elimination at a junction culminates when the branches of losing axons atrophy, detach, form retraction bulbs, and permanently withdraw from muscle fibers, leaving the adult pattern of single innervation (Riley 1977; Balice-Gordon and others 1993; Gan and Lichtman, 1998; Keller-Peck and others 2001).

Is Neural Activity a Mediator or a Modulator of Neuromuscular Synaptic Competition?

The structural and functional changes in the terminal arbor of competing inputs lead to the question of how a target cell discriminates among inputs that are apparently similar in terms of size and strength. Although most evidence supports a role for activity as a modulator of synaptic competition, several lines of evidence suggest that the relative activity of inputs is more salient than the total amount of activity. Blockade of action potentials in

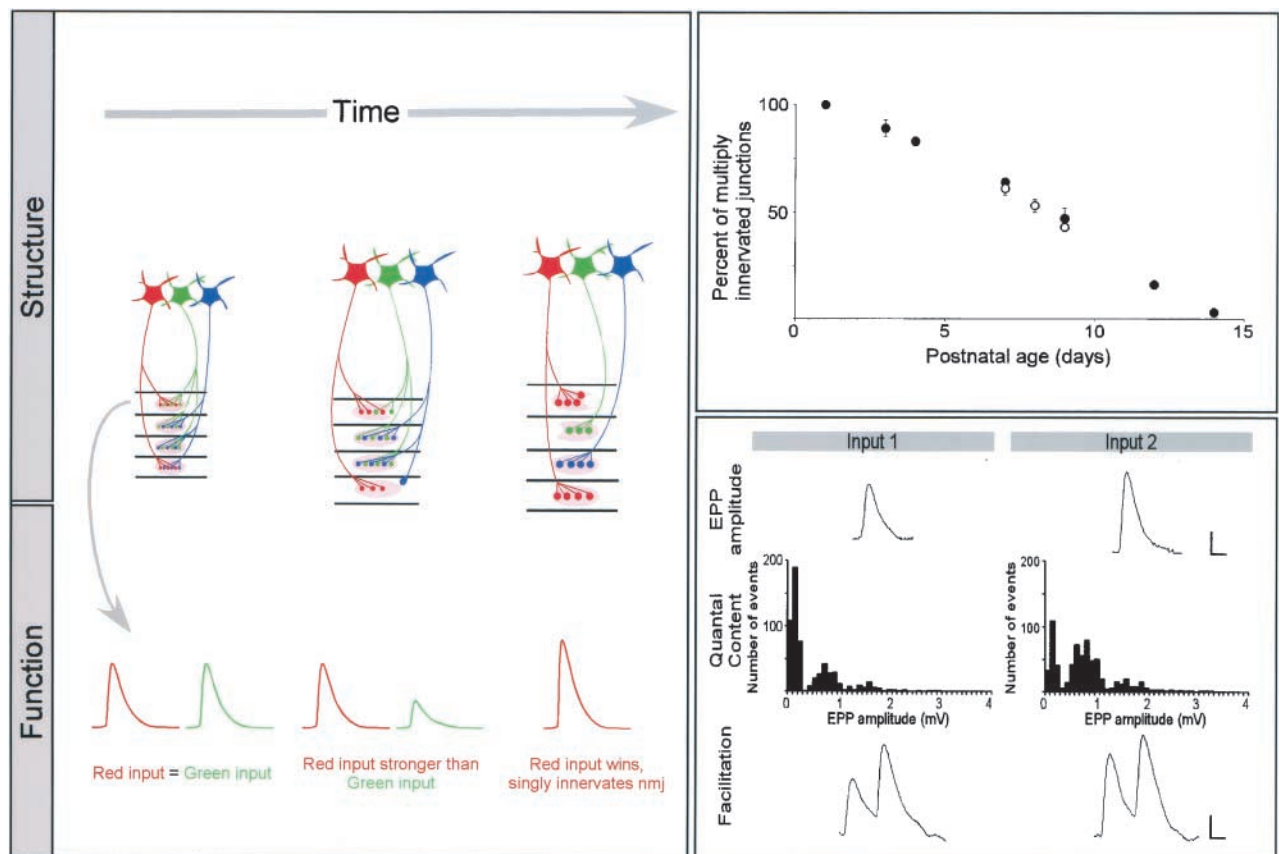


Fig. 3. Changes in synaptic structure and strength during neuromuscular synapse elimination. *Left*, At multiply innervated neuromuscular junctions (nmj), each axon's terminals (red, green, blue) are extensively intermingled over postsynaptic acetylcholine receptor (AChR) clusters (pink) in muscle fibers (black lines). Over time, all but one axon is eliminated, resulting in the mature pattern of single innervation of each junction. As synapse elimination occurs, there are structural changes within each synapse, involving the loss of some AChR regions and perhaps also the capture of synaptic territory by winning inputs. Functional changes in synaptic strength (*bottom*) are also apparent. *Right, Top*, Both anatomical (filled circles) and physiological (open circles) measures show that the transition from multiple to single innervation occurs between P0 and P14. In the soleus muscle, in this example, at birth, all neuromuscular junctions are multiply innervated ($100\% \pm 1\%$), by P7, more than half are multiply innervated ($64\% \pm 2\%$), and few junctions remain multiply innervated by P14 ($3\% \pm 1\%$). *Right, bottom*, Measures of the relative strength of competing inputs can be obtained by measuring the relative sizes of the endplate potentials generated in muscle fibers upon stimulation of each input (amplitude, input 1: 0.23 mV; input 2: 0.37 mV) and by determining the quantal content of each input by the method of failures (histograms; quantal content, input 1: 0.5; input 2: 1.4). Responses to paired pulse stimulation at 10 msec i.p.i. show that neurotransmitter release probability differs among these inputs (facilitation ratio, input 1: 1.4; input 2: 1.0). In the example illustrated here, input 1 had a smaller epp amplitude, a lower quantal content, and a higher facilitation ratio and thus a lower neurotransmitter release probability compared to input 2. Data after Kopp and others (2000). Scale = 0.1 mV, 5.0 msec for A, C, D.

skeletal muscle and nerve, or in nerve alone via a TTX-impregnated cuff, slows the period of developmental synapse elimination (Thompson and others 1979; Ribchester and Taxt 1983; Ribchester 1993), whereas increasing action potential activity with stimulation seems to accelerate this process at neuromuscular junctions in vivo (O'Brien and others 1978; Thompson 1983) and in vitro (Magchielse and Meeter 1986; Nelson and others 1993). This work suggested that synapse elimination is modulated by activity, but it did not rule out the possibility that activity was generally required for synaptic maturation. Work by Thompson in which neonatal rat muscles were chronically stimulated at 1 and 100 Hz showed that the pattern of muscle activation, rather than the total amount of activation, affected synapse elimination (Thompson 1983, 1985). Differential stimulation experiments suggested that more active inputs can dis-

place less active inputs from muscle fibers (Ribchester and Taxt 1983; Ridge and Betz 1984), although qualitatively similar experiments also suggest that inactive motor axons have a competitive advantage over more active axons (Callaway and others 1987; Ribchester 1993).

These and other experiments suggest that, although the overall amount of activity seems to affect the overall rate of synapse elimination, the relative activity among competitors appears to determine the outcome of competition. Inactive or less active inputs would release less neurotransmitter than more active competitors and may as a consequence down-regulate synaptic release machinery, resulting in a further decrease in release probability by weak inputs. Postsynaptic AChRs may then in some cases become depleted under low probability sites in a step-wise fashion, followed by the loss of overlying presynaptic terminal regions. Active inputs may emerge

as winners of competition by maintaining a high quantal content, by maintaining a high release probability, by capturing territory vacated by losing axons, or perhaps also by actively displacing inactive or weakened inputs. These factors may in turn prevent the depletion of postsynaptic AChR receptors, preserving synaptic area and strength. A similar modulation of presynaptic release mechanisms, coupled to changes in postsynaptic neurotransmitter receptor density, may account for structural and functional plasticity commonly observed at neuron-neuron synapses in the developing and mature brain (cf. Quinlan and others 1999; Shi and others 1999).

If all of the motor neurons innervating a muscle fiber were firing relatively synchronously, that muscle fiber may not be able to discriminate among inputs and thus maintain all inputs. On the other hand, if activation of postsynaptic muscle fibers were de-synchronized, as might occur if one motor neuron input was more active than another, the postsynaptic muscle fiber may maintain the more active input and initiate the removal of less active ones. This possibility was tested by manipulating the activation of small regions of AChRs at singly innervated adult neuromuscular junctions (Balice-Gordon and Lichtman 1994). When AChRs in a small region of a junction were blocked by focal application of α -bungarotoxin, blocked receptors were observed to disappear over several days, and nerve terminals overlying the blocked AChRs were subsequently withdrawn. However, uniform blockade of all postsynaptic AChRs did not induce any loss of AChRs or nerve terminals. Thus, when pre- and postsynaptic activity are not temporally correlated, silent synaptic sites are destabilized.

Cangiano and colleagues (Busetto and others 2000) recently demonstrated that synapse elimination was slowed following synchronous stimulation of inputs to newly formed, ectopic synapses in rat muscle. Multiple innervation was maintained when endogenous motor axon activity was blocked by a tetrodotoxin-impregnated cuff, and synchronous stimulation of axons was imposed distal to the block, regardless of the overall level of stimulation. Interestingly, if synchronous stimulation was imposed in the absence of nerve block, thus allowing natural action potential activity to occur in addition to imposed activity, the extent of multiple innervation was the same as in unmanipulated control animals. This result suggests that even a small amount of de-synchronized activity may be sufficient to trigger synaptic competition. These observations suggest that synchronous activation of inputs, or for that matter synchronous inactivity, can slow competition, and they imply that competition is triggered by asynchronous activity within junctions.

Although collectively the bulk of evidence supports the idea that the relative activity of inputs converging on the same target cell may be a modulator of synaptic competition, recent work from Ribchester and colleagues (Costanzo and others 2000) showed that electrically silent inputs can displace other electrically silent inputs during the neuromuscular synapse elimination that occurs during reinnervation of adult muscle. This result

suggests that neither activation of postsynaptic muscle fibers nor asynchronous activity is required for competition to occur. However, the effects of paralysis induced in this study, which can affect the growth and sprouting as well as withdrawal of inactive axons, complicate the interpretation of these experiments. Despite this limitation, this work highlights the important idea that activity may be a modulator, rather than a mediator, of competition. Although the available evidence argues that the relative pattern of activity impinging at neuromuscular junctions is a key determinant of the outcome of competition, it will be important to determine whether activity is instructive or permissive for synaptic competition, during development as well as during reinnervation of adult junctions.

Motor Unit Activity during the Developmental Period of Synaptic Editing

Despite these experimental insights, little information exists about naturally occurring motor neuron firing patterns during synapse elimination, how these patterns change over time, and how these changes affect the emergence of single innervation. This important descriptive information could then be used to shape experimental manipulations that might shed light on the role of activity in synaptic competition. To begin to address this, we monitored the motor behavior of mouse pups and found that newborn mice stand within their nest for prolonged periods of time, moving intermittently with swimming-like movements to change positions within the nest or during nursing. We thus decided to analyze motor neuron firing in the soleus muscle, which is generally active during stance. We recorded the activity of small numbers of motor neurons in awake, behaving neonatal mice using single motor unit electromyography techniques (Fig. 4; Personius and Balice-Gordon 2001). Electromyography records the compound muscle fiber action potential resulting from the activation of the total number of muscle fibers innervated by a single motor neuron. By recording from a small number of motor units from awake, behaving mice, their relative activity could be compared over time.

Micro- and fine-wire electrodes were implanted in the soleus muscle, and stable spontaneous single motor unit activity was obtained from awake animals for several hours (Fig. 4). Because the spatial relationship between the electrode and each active motor unit is unique, we discriminated different motor units based upon their electrical signatures. The soleus muscle is made up of ca. 700 muscle fibers innervated by ca. 20 motor neurons (Fladby 1987), and muscle fibers are innervated by 2 to 6 motor neurons at birth. Thus, a high degree of convergent innervation exists during the first days after birth, so that a pair of discriminated motor units are likely to coinnervate at least some of the same muscle fibers. Despite this convergence, each motor unit's activity will produce a unique signature, since no two motor neurons innervate precisely the same group of muscle fibers (cf. Brown and others 1976; Keller-Peck and others 2001).

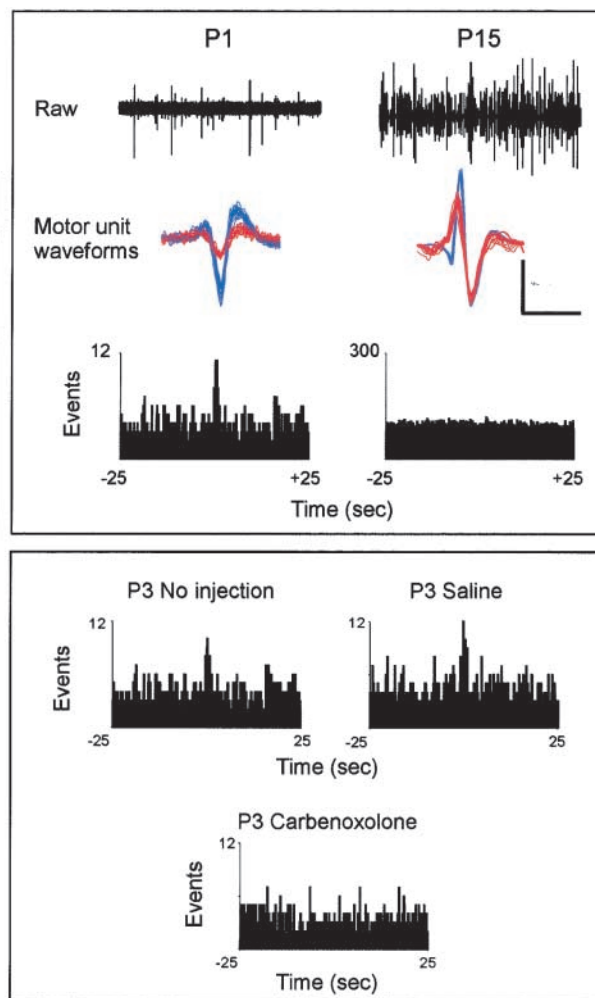
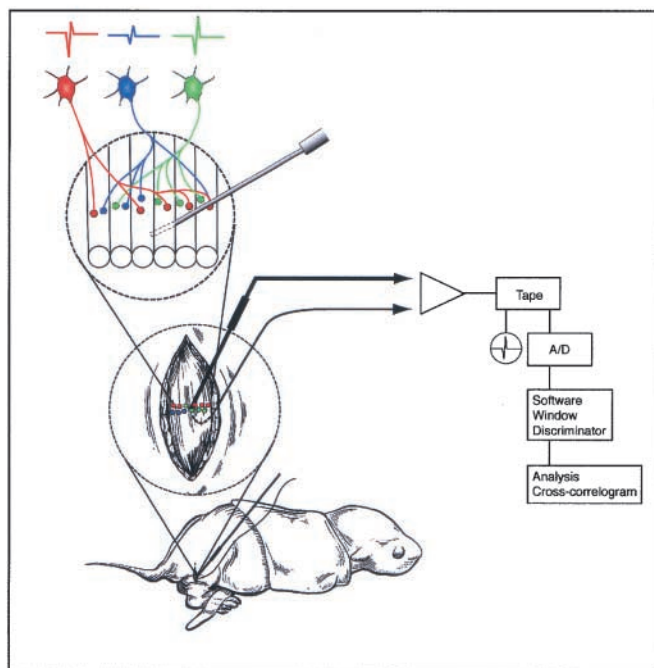


Fig. 4. Single motor unit recordings from awake, behaving neonatal mice. *Left*, P0 to P15 mouse pups were anesthetized and placed in a Kimwipe and dental wax restraint that allowed pups to stand, but not crawl away from, the recording apparatus. The soleus muscle was exposed, and recording electrodes were inserted longitudinally into the muscle belly near the endplate band. Computer-assisted acquisition began as mice recovered from anesthesia and tried to move spontaneously. The activity of different motor units (red, blue, and green) was discriminated by recording the compound muscle fiber action potential (red, blue, and green waveforms) whose shape is dependent upon the spatial relationship between muscle fibers and the electrodes. *Right, upper*, Raw EMG recordings from the soleus muscles of a P1 and P15 mouse. An increase in overall motor unit activity is apparent. Below each raw trace is an overlay of 15 waveforms from each of two discriminated motor units from these records. Additional motor units are present in the raw recording. (Scale bar for raw traces, 0.12 mV, 3 seconds; for color overlay, 0.12 mV, 2 milliseconds). Cross-correlogram analyses with clear peaks around time 0 seconds indicated that the activity of pairs of motor units was temporally correlated at P0, but not at P14. *Right, bottom*, Pharmacological blockers of gap junctions abolished correlations seen in P3 or saline-injected pups, as indicated by a flat cross-correlogram in a representative comparison of two motor units from a P3 mouse treated with carbenoxolone. Data after Personius and Balice-Gordon (2001).

By recording the activity of discriminated motor neurons over several hours, we found that at birth, motor unit firing frequency was quite low (< 1 Hz), and firing frequency did not change from P0 to P9. Motor unit firing frequency increased to 3 to 4 Hz by P14 to P15, and motor units were observed whose firing frequencies approached those reported in freely moving adult rats (Hennig and Lomo 1985; Eken 1998). Because 50% of muscle fibers are singly innervated by P9, this observation suggests that synapse elimination occurs in a milieu of surprisingly low overall activity. Despite the low levels of motor neuron activity at birth, a ca. 16-fold variation in firing frequency was observed at each age between P0 and P9. This large disparity in the level of

motor neuron activity that exists throughout the period of neonatal synapse elimination raises the possibility that two motor neurons convergently innervating the same muscle fiber would likely have quite different levels of activity.

We then examined the temporal relationship of the activity of random pairs of motor units recorded from the same muscle using standard cross-correlogram analysis. Cross-correlograms were generated over periods of ± 25 seconds centered on the firing of one motor unit using 100 millisecond bins. A sharp peak in the cross-correlogram indicates that the activity of the two units is correlated (Fig. 4). From birth to P2, ca. 2/3 of motor units were observed to be temporally correlated

with at least one other discriminated motor unit. By P8 to P9, only about 10% of motor units were temporally correlated, and no correlations were observed in motor units recorded from P14 to P15 mice. The strength of correlation was similar throughout neonatal development, with correlated motor neurons being 3 to 9 times more likely to fire together within a 100 millisecond window than would be expected if the two neurons fired randomly relative to each other. Similar observations of motor unit activity in embryonic and neonatal hindlimb muscles have been reported by Cangiano and colleagues (Buffelli and others 2000). The time scale of correlations was relatively broad, with motor units tending to fire between 100 milliseconds and 1 to 2 seconds of each other, as indicated by the width of the peak in the cross correlogram (Fig. 4). This suggests that the relevant temporal window in which relative activity patterns are compared at neuromuscular junctions is quite broad, as has been observed previously in the retina (Wong and others 1993, 1998). However, in retinotectal neurons *in vivo* (Zhang and others 1998) and dissociated hippocampal neurons *in vitro* (Bi and Poo 1998), the temporal window in which presynaptic activity can modulate synaptic strength is only ca. 20 milliseconds. Thus, the biologically relevant window for temporal correlation seems to be circuit dependent.

Our recent work suggests that motor neuron firing during the period of synapse elimination is initially relatively synchronous and that synchrony disappears as synapse elimination progresses. This raises two related questions: first, what mechanisms underlie this progressive change in motor neuron firing, and second, what is the relationship between this change and the mechanisms driving synaptic competition? There are several possible mechanisms that may modulate motor neuron firing patterns, including the intrinsic excitability of motor neurons, local chemical synaptic connections that excite or inhibit motor neurons, descending cortical inputs, the ingrowth of sensory afferents, and gap junctional communication via electrical synapses that interconnect motor neurons (Fig. 5). Motor neurons within a motor pool are extensively dye- and electrically coupled from birth until P7 (Fulton and Walton 1986; Walton and Navarette 1991; Chang and others 1999). By treating P3 to P4 mice with carbenoxolone, a pharmacological agent that blocks gap junctions, we asked whether gap junctional coupling among motor neurons may shape their firing and thus influence the activity impinging on neuromuscular junctions (Personius and Balice-Gordon 2001). In carbenoxolone-injected mice, no motor units were found to be temporally correlated, whereas about half of the motor units were temporally correlated in saline-injected littermates (Fig. 4). The gap junction blocker used did not result in significant differences in the overall pattern of motor unit activity or firing frequency. These experiments suggest that gap junctional coupling may at least in part underlie the temporal correlations in motor neuron activity observed around the time of birth and that the loss of gap junctional coupling may underlie the loss of correlated motor unit activity seen after P9.

It remains to be determined, however, whether the changes we observed in motor neuron firing patterns mediate or modulate synaptic competition. Preliminary experiments suggest that animals lacking a particular gap junction protein, connexin 40 (Cx40), whose expression is developmentally regulated in motor neurons (Chang and others 1999), undergo synapse elimination several days earlier than wild-type littermates. Motor neuron and muscle fiber number, which may affect the extent of multiple innervation, are unchanged in mutant compared to wild-type mice. We are presently studying how gap junctional coupling and motor neuron activity are affected in these and other connexin mutant mice (Q Chang, K Personius, K Bittman, and R Balice-Gordon, unpublished observations). This approach may be useful to address the hypothesis that the loss of temporally correlated activity among motor neurons triggers synaptic competition, leading to elimination of the least active inputs (Fig. 5). Together this work suggests that the mechanisms that shape motor neuron firing patterns, including but not limited to gap junctional coupling, may ultimately determine the onset and the outcome of synaptic competition.

A paradox that may be more apparent than real is that those motor neurons that successfully capture the largest number of muscle fibers (the largest motor units) are generally those that are the least active: they are recruited last during movements (Henneman and others 1985). This has been used to bolster the argument that inactive motor inputs to junctions have a competitive advantage (Ribchester and Taxt 1983; Callaway and others 1987; Nelson and others 1993; Ribchester 1993). A recent theoretical treatment suggested that less active motor neurons may lose less synaptic territory based on a metabolic advantage conferred by their size and that any competitive advantage conferred by a higher amount of activity early in synapse elimination seemed to be offset by the relatively greater synaptic efficacy of less active motor neurons later in the process (Barber and Lichtman 1999). An alternative explanation may be that, despite the 16-fold variation in motor unit activity observed in EMG recording experiments (Personius and Balice-Gordon 2001), the largest motor units do not emerge as the least active until after single innervation is established (Stollberg 1995). However, the motor units that start out the largest early in postnatal life seem to lose the most muscle fibers during synapse elimination (Brown and others 1976; Balice-Gordon and Thompson 1988). Thus, it seems plausible that the least active motor units ultimately lose more synaptic targets as a result of synaptic competition than more active motor units.

Although progress in the field of neuromuscular synapse elimination has shed light on the mechanisms generally underlying synaptic plasticity, many questions remain to be addressed. How are structural changes in the deployment of different inputs within a neuromuscular junction related to the progressive changes in their synaptic strength that are a hallmark of competition? Studies that combine structural observations in transgenic mice expressing GFP variants in motor axons,

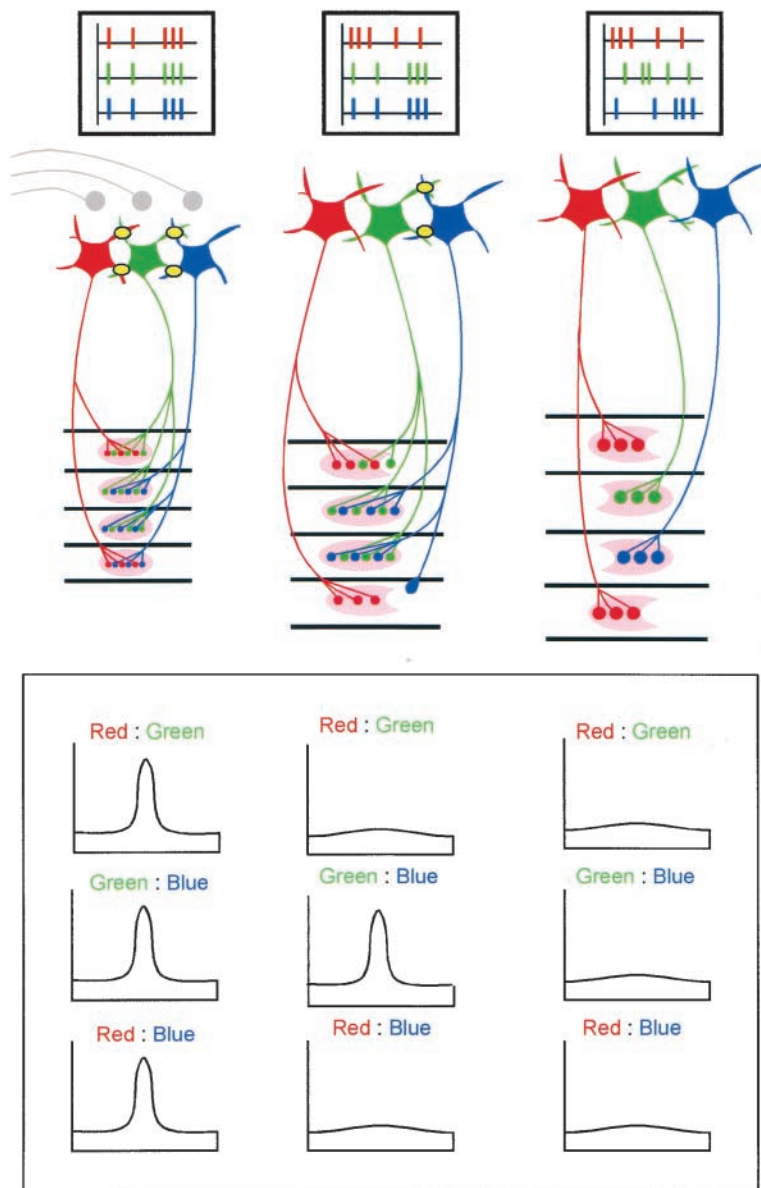


Fig. 5. Patterns of neural activity modulate synaptic editing at developing neuromuscular synapses. During late embryonic and early neonatal development, when each muscle fiber is multiply innervated (*left*), motor neuron action potential activity may be temporally correlated (*left box*), as evidenced by peaks in cross-correlograms comparing the activity of different inputs (*bottom*). Several days after birth, correlated activity among some motor neurons begins to disappear (*middle box*, red neuron). The loss of correlated activity, evidenced by the absence in cross-correlogram peaks, may trigger synaptic competition (*middle*). By 2 to 3 weeks after birth, each muscle fiber is innervated by one motor neuron (*right*) and motor unit activity is no longer correlated (*right box*). Although the pattern and amount of motor neuron activity may change during postnatal life, in the example illustrated here, the pattern of the red motor neuron is altered, but the total amount of neural input remains constant. The amount and pattern of motor neuron activity may be shaped by the ingrowth of descending cortical and brainstem inputs, by the maturation of local spinal cord circuitry and sensory feedback from muscle (both represented by gray lines), and/or by gap junctional coupling among motor neurons (yellow ovals). A key issue in the field that remains to be resolved is whether differences in motor neuron firing patterns mediate or modulate synaptic competition.

optical measures of vesicle recycling using FM-143, and physiological measures of synaptic strength should allow the relationship between structural and functional synaptic changes to be resolved in the near future. How are the mechanisms driving synapse elimination controlled locally within each neuromuscular junction, in light of the fact that a neuron propagates identical patterns of activity to all of its branches? Direct observations of the timing of synapse elimination show that each of its axon branches and terminals are at different stages of the process at different times (Keller-Peck and others 2001). One possible explanation is that, because each of a neuron's branches is competing with a unique cohort of other neuronal branches that are likely to differ widely in their activity, the milieu of neural activity is unique at each junction, and this milieu modulates synapse elimination differentially across the terminals of an axon. How do changes in motor neuron activity patterns lead

to the progressive disparity in synaptic strength among inputs that is a hallmark of synaptic competition? Although heterosynaptic interactions have been explored at immature synapses in *Xenopus* spinal cord neuron-myoblast cultures (Lo and Poo 1991; Dan and Poo 1992) and in CNS synapses in vitro (Bi and Poo 1998), little information exists on the molecular or cellular nature of these interactions. Signals that destabilize inactive synapses as well as signals that protect active ones have yet to be identified (Lichtman and Colman 2000). Thus, much work remains to elucidate the cascade of molecular events linking motor neuron activity to changes in synaptic strength and structure. Similar mechanisms may account for the structural and functional plasticity commonly observed at neuron-neuron synapses in the developing and mature brain. If changes in synaptic strength within the CNS also result in the permanent loss of ineffective inputs, this would provide a mechanism by

which changes in synaptic function would become permanently etched in neural circuitry.

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