

Synaptic Segregation at the Developing Neuromuscular Junction

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Throughout the developing nervous system, competition between axons causes the permanent removal of some synaptic connections. In mouse neuromuscular junctions at birth, terminal branches of different axons are intermingled. However, during the several weeks after birth, these branches progressively segregated into nonoverlapping compartments before the complete withdrawal of all but one axon. Segregation was caused by selective branch atrophy, detachment, and withdrawal; the axon branches that were nearest to the competitor's branches were removed before the more distant branches were removed. This progression suggests that the signals that mediate the competitive removal of synapses must decrease in potency over short distances.

Competitive synaptic rearrangements change the number and strength of axonal connections with target cells in many parts of the nervous system (1). At each neuromuscular junction, for example, motor axons compete until all but one are removed (2). By iontophoretic application of lipophilic dye (3, 4), we observed synaptic competition between individual axon terminals at the developing neuromuscular junction of the mouse.

We began by looking at neuromuscular junctions during the second postnatal week [postnatal days 9 and 10 (P9-10)], when most muscle fibers (88%) were singly innervated and the rest (12%) were innervated by two axons (5). At the singly innervated junctions ($n = 242$; 20 muscles), the entire postsynaptic receptor territory was overlain by one axon, as observed by staining with lipophilic dyes or with nerve-specific antibodies (6). However, at the remaining 12% of the junctions, a labeled axon occupied only part of the receptor territory (Fig. 1, A through D). Subsequent staining with antibodies to neurofilaments or with tetanus toxin (6) showed that, in each of these cases ($n = 33$), the remaining part of the neuromuscular junction was innervated by another axon (Fig. 1A). In these dually innervated junctions, the labeled terminals of each individual axon occupied a variably sized but contiguous region extending from one edge of the junction toward the center (Fig. 1, A through D). Thus, in the second postnatal week, each axon's synaptic territory is confined to a separate region of the junction.

By contrast, at earlier stages (P1-3), a single axon typically occupies multiple small regions that are distributed throughout the neuromuscular junction (Fig. 1, E and F). As

expected, the sites that are occupied by different axons are intermingled ($n = 26$) (Fig. 2A) (7). The difference in the distribution of axon terminals between P1-3 and P9-10 could mean that the areas that are occupied by different axons segregate from each other over time. Alternatively, a small number of junctions may already be segregated at birth, and these junctions may retain multiple innervation longer than intermingled ones. To explore these possibilities, we used different lipophilic dyes to separately label each axon that innervated the same neuromuscular junction, and we measured the separation between the terminal areas that were occupied by the two competing axons (8) (Fig. 2, A through C). We found no junctions at birth in which axons had completely nonoverlapping territories. Thereafter, both the absolute separation (the distance between two centroids) and the relative separation [the distance normalized to the total length of the junction (Fig. 2E)] of the terminal areas gradually increased. In most cases, the separation was only partial at P3-4 (Fig. 2, B and E) and was complete by P7-10 (Fig. 2, C and E). By P16-17, when very few junctions (1.4%) were still multiply innervated, axon territories in all of the multiply innervated junctions (8 out of 8) showed a high degree of separation with an uninnervated gap in between (9) (Fig. 2, D and E). Therefore, neuromuscular junctions become progressively compartmentalized as development proceeds.

To learn how this segregation of terminals occurred, we examined multiply innervated junctions at P5-7 before segregation was complete in all junctions ($n = 62$). We found that, at multiply innervated junctions, individual axons often (30 out of 62) possessed two types of branches, thick and thin (Fig. 3, A and B). The thin axon branches were always nearest to or within the territory occupied by the competing axon, whereas the thickest branches were located on the perimeter of the junction at the great-

monocular deprivation. A >100-fold saturating dose of diazepam was determined by recording GABA_A responses in visual cortical slices (16), then infused to ensure adequate drug diffusion in vivo. Local rescue was achieved with low-flow osmotic minipumps (0.5 μ l/hour; Alzet 1007, Alza) containing drug or vehicle solution connected to cannulae (30 gauge) that were stereotactically implanted into one hemisphere under sterile surgical conditions 2 days before eyelid suture. Control experiments demonstrated that continuous infusion of TTX or dye solutions from osmotic minipumps could be restricted to ipsilateral visual cortex without disturbing the gross morphology of the binocular zone at least 1.5 mm away from the cannula tip. Drug was infused continuously for 4.5 hours at a high rate (3 μ l/hour) to determine the spread of diazepam. In agreement with its known rapid breakdown in vivo [L. A. Berrueta, B. Gallo, F. Vicente, *J. Pharm. Biomed. Anal.* **10**, 109 (1992)], diazepam was not detectable after 1 week of pumping at low rates. A diazepam detection assay was developed using reversed-phase HPLC (UV detection at 240 nm) after organic extraction: Brain tissue (~20 mg) was sonicated with ethyl acetate (400 μ l) and centrifuged (8000g, 15 min), the supernatant extracted on a mechanical shaker (1 M tris-HCl, pH 9.0, 20 min), and the organic layer evaporated under a gentle N₂ stream. The residue was reconstituted in ethyl acetate (100 μ l) and back-extracted with 6 N HCl (40 μ l); the aqueous phase was adjusted to neutral (3 M tris buffer, pH 11.6) and injected onto an HPLC apparatus. The mobile phase was 50 mM sodium phosphate buffer (pH 6.4) with 38% acetonitrile and 0.1% triethylamine.

21. Similarly weak and variable plasticity was observed in GAD65 KO versus WT mice that had experienced deprivations spanning the entire critical period (P17-36), despite the longer temporal integration and potential additional contribution of anatomical changes to the functional disconnection of deprived eye input (7). CBI = 0.54 ± 0.04 versus 0.41 ± 0.02 ; six KO and four WT mice, respectively ($P < 0.05$, t test).
22. F. Zafra, E. Castren, H. Thoenen, D. Lindholm, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10037 (1991); H. Thoenen, *Science* **270**, 593 (1995); A. K. McAllister, D. C. Lo, L. C. Katz, *Neuron* **17**, 1057 (1996); T. Bonhoeffer, *Curr. Opin. Neurobiol.* **6**, 119 (1996); R. Sala et al., *Eur. J. Neurosci.* **10**, 2185 (1998); L. C. Rutherford, S. B. Nelson, G. G. Turrigiano, *Neuron* **21**, 521 (1998).
23. A. Artola and W. Singer, *Nature* **330**, 649 (1987); M. F. Bear and A. Kirkwood, *Curr. Opin. Neurobiol.* **3**, 197 (1993); S. M. Dudek and M. J. Friedlander, *Neuron* **16**, 1097 (1996); K. D. Miller, *ibid.* **17**, 371 (1996).
24. T. K. Hensch and M. P. Stryker, *Science* **272**, 554 (1996); T. K. Hensch et al., *J. Neurosci.* **18**, 2108 (1998).
25. J. DeFelipe, *Cereb. Cortex* **3**, 273 (1993); Y. Gonchar and A. Burkhalter, *ibid.* **7**, 347 (1997); Y. Kang, T. Kaneko, H. Ohishi, K. Endo, T. Araki, *J. Neurophysiol.* **71**, 280 (1994); J. F. M. Van Brederode and W. J. Spain, *ibid.* **74**, 1149 (1995); H. J. Luhmann and D. A. Prince, *ibid.* **65**, 247 (1991); Y. Komatsu, *Dev. Brain Res.* **8**, 136 (1983).
26. R. K. Carder, S. S. Leclerc, S. H. C. Hendry, *Cereb. Cortex* **6**, 271 (1996); Y. Kawaguchi, *J. Neurophysiol.* **78**, 1743 (1997); D. Parra, A. I. Gulyas, R. Miles, *Neuron* **20**, 483 (1998); Y. Kawaguchi and T. Shindou, *J. Neurosci.* **18**, 6963 (1998); Z. Xiang, J. R. Huguenard, D. A. Prince, *Science* **281**, 985 (1998); G. Tamas, P. Somogyi, E. Buhl, *J. Neurosci.* **18**, 4255 (1998).
27. A. Gulyas, R. Miles, N. Hajos, T. F. Freund, *Eur. J. Neurosci.* **5**, 1729 (1993); E. Buhl, K. Halasy, P. Somogyi, *Nature* **368**, 823 (1994); I. Soltesz, D. K. Smetters, I. Mody, *Neuron* **14**, 1273 (1995); R. Miles et al., *ibid.* **16**, 815 (1996); G. Tamas, E. H. Buhl, P. Somogyi, *J. Physiol. (London)* **500**, 715 (1997).
28. Y. Kawaguchi and Y. Kubota, *Cereb. Cortex* **7**, 476 (1997); L. S. Benardo, *J. Physiol. (London)* **476**, 203 (1994); Y. Kawaguchi, *Exp. Brain Res.* **88**, 33 (1992); S. Sugita, S. W. Johnson, R. A. North, *Neurosci. Lett.* **134**, 207 (1992); M. Segal, *Brain Res.* **511**, 163 (1990); A. M. Thomson and D. C. West, *Soc. Neurosci. Abstr.* **23**, 379.7 (1997).
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est distance from the competing axon's territory. Some thin branches (6 out of 30) had swollen ends (arrows in Fig. 3, A and B) that were reminiscent of "retraction bulbs," which were previously observed during the final stages of synapse elimination when an axon withdraws completely from a junction (7, 10) (see also Fig. 4B). Neurofilaments were detectable in the thick branches but not in the thin branches (insets in Fig. 3, A and B). The thick branches were closely apposed to the postsynaptic membrane. In contrast, the thin branches were often several microns above the postsynaptic receptor plaque (Fig. 3C). Together, these data suggest that branches of the same axon within the same neuromuscular junction behave differently; whereas the thin branches undergo retraction, the thick branches remain intact. These observations also suggest that segregation of competing axon terminals is caused by a progressive process of branch atrophy, detachment, and withdrawal that begins with the branches nearest the territory dominated by a competing axon.

Given the intermingling of axons throughout all regions of neuromuscular junctions at birth, the complete absence of overlap in the second postnatal week implies that both inputs (the eventual winner and loser) lose branches in many junctions. We observed doubly innervated junctions in which both axons possessed thin branches in the territories dominated by their competitors and thick branches at a distance from the other axon's terminals (Fig. 2B, arrows). Therefore, at some junctions (at least), both axons appear to be relatively effective competitors that are capable of removing some branches of the other axon.

After segregation, branches of one of the two inputs must have continued to withdraw because the average percentage of multiply innervated junctions changed from 42% at P7 to 4% at P12 (5) and to 0% in adult animals (in this study, 0 out of 354). At most of the segre-

gated multiply innervated junctions (77 out of 90) at P7-12, we observed no distinctions between the axons that would suggest which one

would eventually be eliminated. Some segregated junctions (13 out of 90), however, did show characteristics that may be related to the

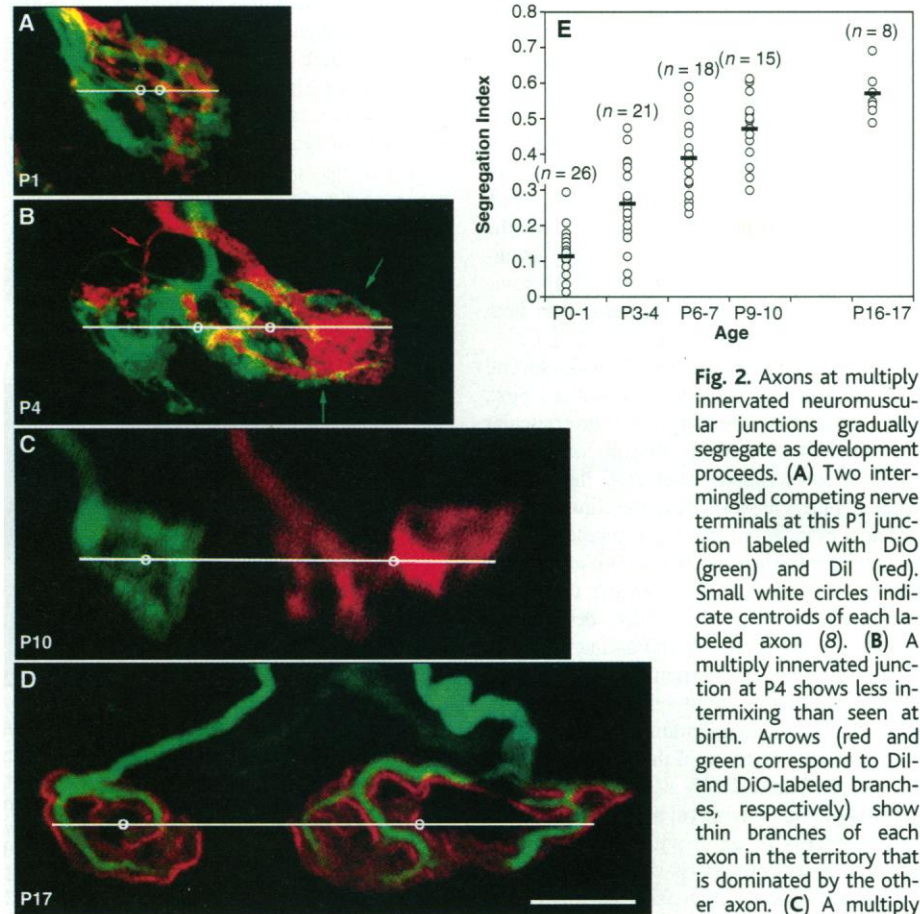


Fig. 2. Axons at multiply innervated neuromuscular junctions gradually segregate as development proceeds. (A) Two intermingled competing nerve terminals at this P1 junction labeled with DiO (green) and Dil (red). Small white circles indicate centroids of each labeled axon (8). (B) A multiply innervated junction at P4 shows less intermingling than seen at birth. Arrows (red and green correspond to Dil- and DiO-labeled branches, respectively) show thin branches of each axon in the territory that is dominated by the other axon. (C) A multiply innervated junction at

P10 in which the two inputs are separated by an uninervated gap (at most junctions at this age, however, the inputs are separate but juxtaposed). (D) At P17, two competing axons at this rare remaining multiply innervated junction show a large separation between two inputs. In this case, both axons were stained with an antibody to neurofilament (green) and the postsynaptic AChRs were labeled with fluorescently tagged α -bungarotoxin (red). (E) The segregation index (8) increases through early postnatal life. Each circle represents an individual junction; horizontal bars are the means of the segregation index. Scale bar in (D), 10 μ m [shows scale for (A) through (D)].

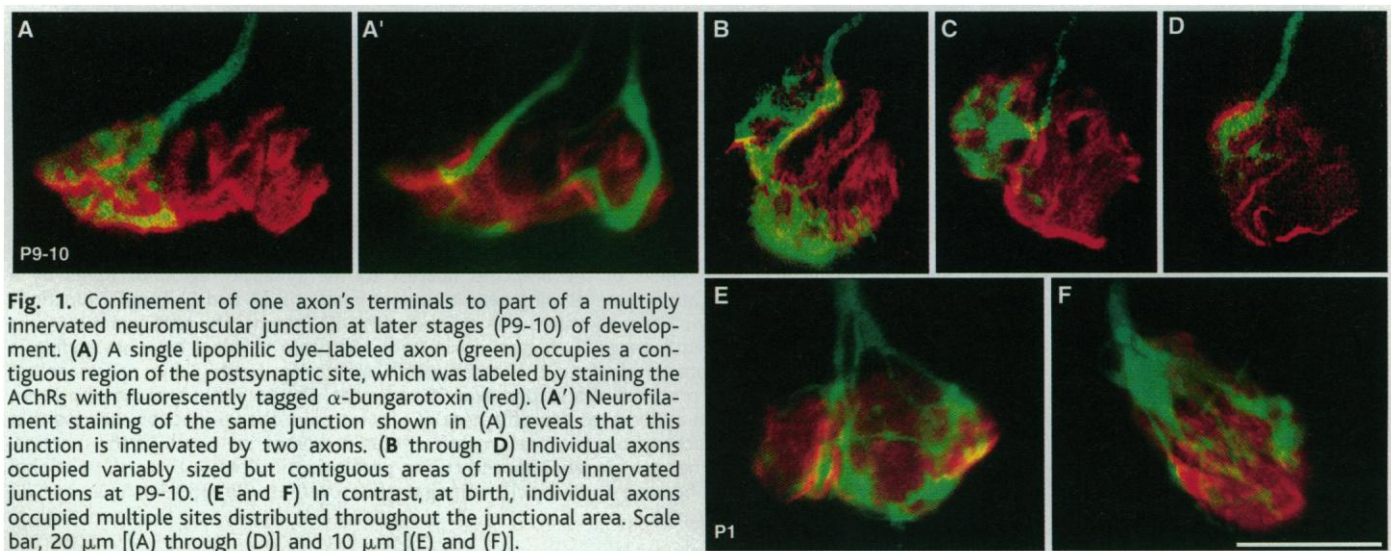


Fig. 1. Confinement of one axon's terminals to part of a multiply innervated neuromuscular junction at later stages (P9-10) of development. (A) A single lipophilic dye-labeled axon (green) occupies a contiguous region of the postsynaptic site, which was labeled by staining the AChRs with fluorescently tagged α -bungarotoxin (red). (A') Neurofilament staining of the same junction shown in (A) reveals that this junction is innervated by two axons. (B through D) Individual axons occupied variably sized but contiguous areas of multiply innervated junctions at P9-10. (E and F) In contrast, at birth, individual axons occupied multiple sites distributed throughout the junctional area. Scale bar, 20 μ m [(A) through (D)] and 10 μ m [(E) and (F)].

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final stages of synaptic competition [the relatively low incidence of observation of these characteristics may mean that the final events of branch withdrawal are rapid (11)]. At these junctions, one axon was thinner than the other axon (Fig. 4A). In addition, in some junctions, the postsynaptic acetylcholine receptor (AChR) density associated with the thin axon was lower than that associated with the thicker axon (Fig. 4A). In some cases, a thin axon possessing a retraction bulb was located near but not apposed to a very dimly stained AChR site, which presumably was previously occupied by the withdrawing axon (Fig 4B). Thus, axons apparently compete even when separated by some distance, resulting in the disassembly of both the pre- and postsynaptic sites.

The studies reported here provide several insights regarding synaptic competition. First, even within the confines of a neuromuscular junction that is ~ 10 to $50\ \mu\text{m}$ long, some branches of a single axon are maintained, whereas others are lost. Thus, the elimination of different branches of an axon is regulated independently at the level of each individual branch. Second, departing axonal branches undergo a stereotyped process of atrophy, detachment, and retraction within a neuromuscular junction. This program of branch removal seems analogous to the final stage of synapse elimination, when the losing axon terminates in a retraction bulb as it withdraws from the end plate (compare Figs. 3 and 4) (7, 10). Therefore, the process of branch removal may illuminate the entire process of axon withdrawal. Finally, the withdrawal process is spatially regulated so that the branches of an axon that are nearest

to the competitor's territory undergo retraction but branches of the same axon several micrometers more distant from the competitor's territory appear unaffected. It is this pattern of distance-dependent loss that leads to the segregation of competing axons. The local regulation of axon branch removal does not support the idea that signals that maintain their strength over distance (such as action potentials) are mediators of synaptic competition (12).

Axons may compete by generating activity-mediated signals that destabilize synaptic sites associated with other inputs (13). Our results indicate that such destabilizing signals would have to be spatially regulated and decrease in potency over distances of $<50\ \mu\text{m}$. The nature

of the spatially localized signals in this process is not known. Experiments in *Xenopus* nerve muscle cocultures suggest that activation at one synaptic site can decrease synaptic efficacy at nearby sites that are occupied by different inputs through a steeply decreasing intracellular Ca^{2+} signal (14). It is therefore possible that a diffusible signal cascade triggered by local calcium entry [for example, through activated AChRs or other Ca^{2+} channels (15)] could initiate (16), or itself be, the spatially decreasing signal at developing neuromuscular junctions. Although such a spatially decreasing signal could in principle explain segregation, it would not necessarily result in the elimination of an input when it had obtained a sufficient

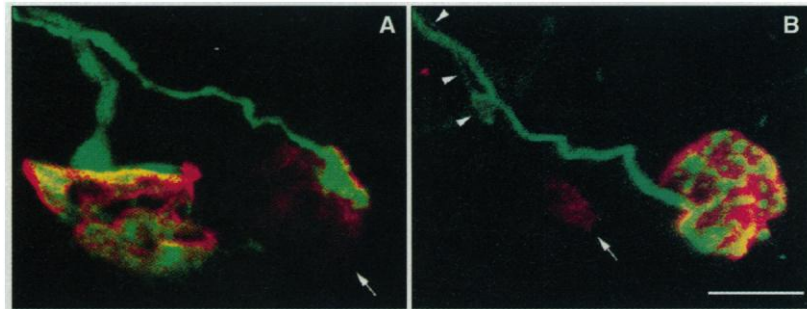


Fig. 4. Synapse elimination continues after segregation. (A) DiO staining of two axons innervating the same neuromuscular junction at P7. The postsynaptic AChRs were labeled with fluorescently tagged α -bungarotoxin (red). The two axons differ in caliber. The area occupied by the thinner axon is smaller and is adjacent to a faintly stained patch of AChRs (arrow). (B) A retracting thin axon (arrowheads) terminates in a retraction bulb, which is near a very low density AChR site (arrow). These receptors were presumably occupied by that axon before its withdrawal. Thus, in development as in synaptic competition in adult muscle (5, 20), the loss of receptors continues after the nerve has vacated a synaptic site. Scale bar, $15\ \mu\text{m}$ (A) and $10\ \mu\text{m}$ (B).

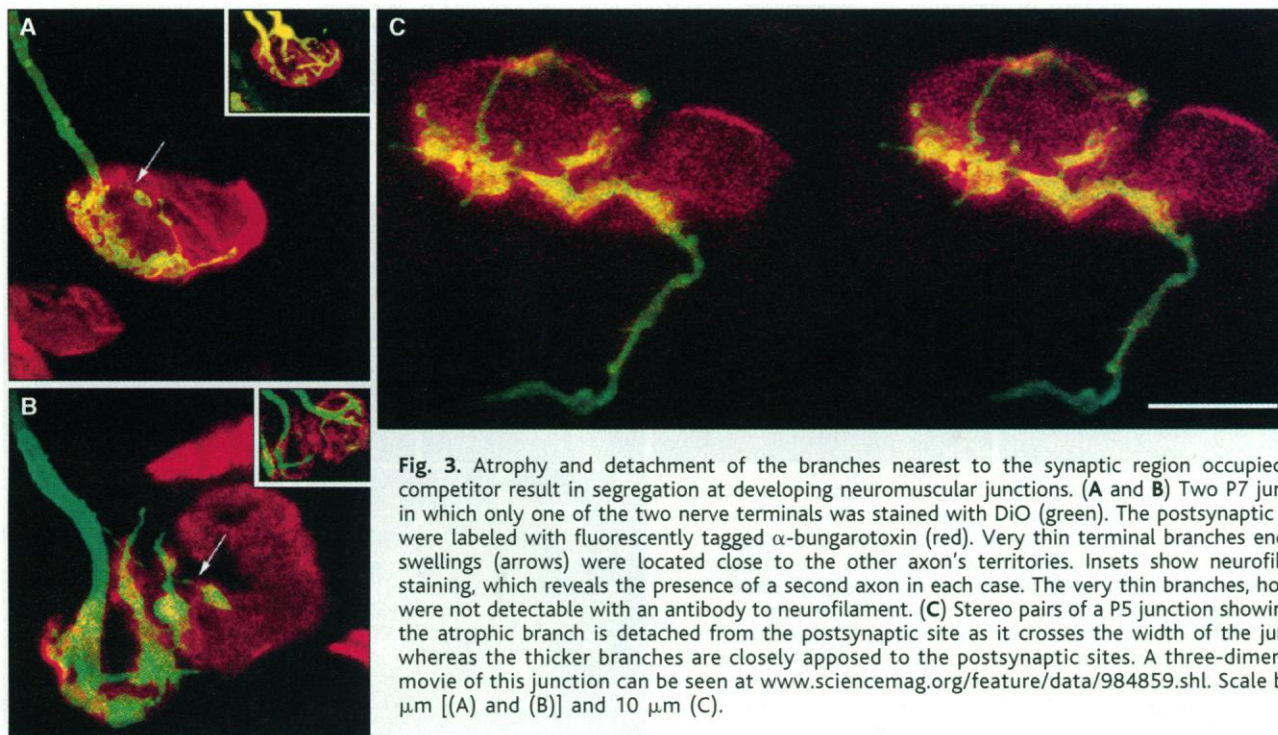


Fig. 3. Atrophy and detachment of the branches nearest to the synaptic region occupied by a competitor result in segregation at developing neuromuscular junctions. (A and B) Two P7 junctions in which only one of the two nerve terminals was stained with DiO (green). The postsynaptic AChRs were labeled with fluorescently tagged α -bungarotoxin (red). Very thin terminal branches ending in swellings (arrows) were located close to the other axon's territories. Insets show neurofilament staining, which reveals the presence of a second axon in each case. The very thin branches, however, were not detectable with an antibody to neurofilament. (C) Stereo pairs of a P5 junction showing that the atrophic branch is detached from the postsynaptic site as it crosses the width of the junction, whereas the thicker branches are closely apposed to the postsynaptic sites. A three-dimensional movie of this junction can be seen at www.sciencemag.org/feature/data/984859.shl. Scale bar, $20\ \mu\text{m}$ [(A) and (B)] and $10\ \mu\text{m}$ (C).

distance from the competitor. Therefore, we presume that the strength or extent of the short-range destabilizing signals increases during synaptic maturation [as synaptic efficacy is increasing (11)] or that the destabilization process is itself incremental (taking longer to remove distant synapses than nearby ones), or that both processes occur. Because the decreasing signals may become totally ineffective when the competitors are sufficiently separated (17), the normal confinement of all the incoming axons to a small AChR plaque may thus be a strategy that encourages strong competition among nearby synapses, which rapidly results in single innervation at developing neuromuscular junctions. Given the existence of competitive synaptic reorganization on neurons (18) and the evidence for the restriction of axonal innervation to parts of a dendritic arbor (19), it seems likely that analogous short-range signals operate throughout the nervous system.

References and Notes

1. D. Purves and J. W. Lichtman, *Principles of Neural Development* (Sinauer, Sunderland, MA, 1985).
2. P. A. Redfern, *J. Physiol.* **209**, 701 (1970); J. K. S. Jansen and T. Fladby, *Prog. Neurobiol.* **34**, 39 (1990).
3. W.-B. Gan and E. R. Macagno, *J. Neurosci.* **15**, 3243 (1995).
4. Neonatal mice (between P0 and P17) were anesthetized with 0.1 ml of sodium pentobarbital. Sternomastoid muscles were then dissected and placed in petri dishes in physiological saline. In some cases, junctional AChRs were labeled for 10 min with tetramethyl rhodamine-conjugated α -bungarotoxin (5 μ g/ml) (Molecular Probes, Eugene, OR). Sharp electrodes (5 to 10 megohms, as measured with 3 M KCl) were backfilled with a 1% solution of 3,3'-diocadecyloxycarbocyanine perchlorate (DiO) (Molecular Probes) in a 100% solution of methylene chloride (Sigma) and positioned on a superficial neuromuscular junction. Depolarizing current (200 ms, 1 to 10 nA, and 1 Hz) was applied for a few seconds until a dye crystal was deposited at the junction. The muscle was then fixed in a 4% solution of paraformaldehyde for 12 hours, over which time the fluorescent DiO lipid labeled many terminals of one motor unit that were distributed over several hundred micrometers. For the labeling of two competing axons at the same neuromuscular junction, two electrodes [each containing 1% solutions of either 1,1'-diocadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes) or DiO in a 100% solution of methylene chloride] were used to deposit dye. In this way, two different subsets of axon terminals, which by chance occasionally converged at the same junction, were labeled. All labeled junctions were imaged with confocal microscopy (Noran Odyssey, Olympus Fluoview, and Bio-Rad MRC1024) with 1.4-numerical aperture objectives, and three-dimensional (3D) reconstructions were generated with the Bio-Rad MRC1024 software.
5. R. J. Balice-Gordon and J. W. Lichtman, *J. Neurosci.* **13**, 834 (1993).
6. Staining of all motor axons [with antibodies against neurofilaments, synaptic vesicles (SV2), and acetylated microtubules] was done similarly as described in (11). For tetanus toxin labeling, the preparation was incubated with a fluorescein isothiocyanate-conjugated recombinant tetanus toxin C fragment (Boehringer Mannheim) for 20 min (1:10 dilution).
7. R. J. Balice-Gordon, C. K. Chua, C. C. Nelson, J. W. Lichtman, *Neuron* **11**, 801 (1993).
8. To measure the separation of two competing axons, we calculated a segregation index as the distance between centroids of the competing axons divided by the total length of the junction, which was measured along a line passing through the two centroids. The centroid (geometric center) of the terminal branches

- of each competing axon was obtained with IP Lab software (Scanalytics, Fairfax, VA). The particular shape of the junction will influence this measure to some degree. For hemicircles that approximate the shapes of competing axon terminals, values >0.39 indicate that the regions are completely segregated.
9. Because of the low incidence of multiply innervated junctions after P12, we used antibody staining instead of lipophilic dyes to label competing axons at P16-17. Competing axons innervating the same junction at this age were traced several hundred micrometers back into nerve bundles to confirm that they were separate axons.
10. D. A. Riley, *Brain Res.* **134**, 279 (1977); *J. Neurocytol.* **10**, 425 (1981).
11. H. Colman, J. Nabekura, J. W. Lichtman, *Science* **275**, 356 (1997).
12. G. S. Stent, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 997 (1973).
13. R. J. Balice-Gordon and J. W. Lichtman, *Nature* **372**, 519 (1994); C. Jennings, *ibid.*, p. 498.
14. Y.-J. Lo and M.-M. Poo, *Science* **254**, 1019 (1991); Y. Dan and M.-M. Poo, *ibid.* **256**, 1570 (1992); S. Cash, Y. Dan, M.-M. Poo, R. Zucker, *Neuron* **16**, 745 (1996).
15. S. Rotzler, H. Schramek, H. R. Brenner, *Nature* **349**, 337 (1991).

16. D. J. Zou and H. T. Cline, *Neuron* **16**, 529 (1996); G.-Y. Wu, R. Malinow, H. T. Cline, *Science* **274**, 972 (1996).
17. M. C. Brown, J. K. S. Jansen, D. Van Essen, *J. Physiol.* **261**, 387 (1976); D. Kuffler, W. Thompson, J. K. S. Jansen, *Brain Res.* **138**, 353 (1977).
18. D. H. Hubel, T. N. Wiesel, S. LeVay, *Philos. Trans. R. Soc. London Ser. B* **278**, 377 (1977); D. Purves and J. W. Lichtman, *Science* **210**, 153 (1980); M. C. Crair, D. C. Gillespie, M. P. Stryker, *ibid.* **279**, 566 (1998); A. A. Penn, P. A. Riquelme, M. B. Feller, C. J. Shatz, *ibid.*, p. 2108.
19. C. J. Forehand and D. Purves, *J. Neurosci.* **4**, 1 (1984); R. Young and E. W. Rubel, *J. Comp. Neurol.* **254**, 425 (1986); M. Bank and S. Schacher, *J. Neurosci.* **12**, 2960 (1992); M. Bravin, F. Rossi, P. Strata, *J. Comp. Neurol.* **357**, 395 (1995).
20. M. M. Rich and J. W. Lichtman, *J. Neurosci.* **9**, 1781 (1989).
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The Role of Far1p in Linking the Heterotrimeric G Protein to Polarity Establishment Proteins During Yeast Mating

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Heterotrimeric guanosine triphosphate (GTP)-binding proteins (G proteins) determine tissue and cell polarity in a variety of organisms. In yeast, cells orient polarized growth toward the mating partner along a pheromone gradient by a mechanism that requires Far1p and Cdc24p. Far1p bound G $\beta\gamma$ and interacted with polarity establishment proteins, which organize the actin cytoskeleton. Cells containing mutated Far1p unable to bind G $\beta\gamma$ or polarity establishment proteins were defective for orienting growth toward their mating partner. In response to pheromones, Far1p moves from the nucleus to the cytoplasm. Thus, Far1p functions as an adaptor that recruits polarity establishment proteins to the site of extracellular signaling marked by G $\beta\gamma$ to polarize assembly of the cytoskeleton in a morphogenetic gradient.

Asymmetric cellular organization or cell polarity is a central feature of morphogenesis and is controlled by both internal and external signals (1). In the yeast *Saccharomyces cerevisiae*, mating pheromones trigger a mitogen-activated

protein kinase (MAPK) signal transduction pathway, culminating in arrest of the cell cycle, changes in gene expression, and altered cell polarity and morphology (2). These responses are initiated by a cell-surface receptor coupled to a G protein. Activation of the pheromone receptor triggers dissociation of the heterotrimeric G protein into subunits G α and G $\beta\gamma$, which in turn signal to downstream effectors to induce cellular responses (3). Cells use a pheromone gradient to locate their mating partner and polarize their actin cytoskeleton toward the site of the highest pheromone concentration (4). Far1p and Cdc24p are necessary for oriented cell polarity: specific alleles of *FAR1* (*far1-s*) and *CDC24* (*cdc24-m*) have been identified which cause a specific mating defect, because

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