Long-term synapse loss induced by focal blockade of postsynaptic receptors

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Focal application *in vivo* of α-bungarotoxin to block neurotransmission in a small region of a neuromuscular junction causes long-lasting synapse elimination at that site. In contrast, blockade of neurotransmission throughout a junction does not cause synapse elimination. These and related experiments indicate that active synaptic sites can destabilize inactive synapses in their vicinity.

THE persistence of memories in humans over many decades argues that experience can cause long-lasting structural modifications in synaptic circuitry. How such modifications occur is poorly understood, partly because of the difficulty of monitoring synaptic connections in the central nervous system over long time periods. Long-term monitoring of synaptic connections is possible however, at the neuromuscular junction, a simple and accessible synapse where structural changes in connections (synaptic plasticity) have been directly observed[1-7].

One well known instance of plasticity at neuromuscular junctions occurs in neonatal life when each junction undergoes a transition from innervation by multiple motor axons to innervation by only one[8,9]. The loss of synapses that underlies this change in connectivity is a protracted process spanning several weeks. In mice, the losing axon at each junction gradually relinquishes synaptic territory, finally withdrawing when all of its synaptic endings have been removed[10]. The removal of presynaptic terminals is matched by a corresponding loss of postsynaptic acetylcholine receptors (AChRs) from the muscle fibre membrane at the same sites[2,11]. The changes in AChR density in the postsynaptic membrane begin before the overlying nerve terminals are removed, suggesting that the postsynaptic cell may be instigating the removal of some of the synapses impinging on it while at the same time maintaining the rest.

One way a developing muscle fibre might maintain some AChR clusters (at sites occupied by one axon) while simultaneously disassembling nearby AChR clusters (at sites occupied by other axons) would be for it to sense differences in the timing of neural activity from the different axons. In particular, if the activity pattern of the set of synaptic terminals derived from one axon (a cartel[12]) was different from the activity pattern of the synapses derived from another axon, then all the receptor regions within a multiply innervated junction would not be synchronously activated. In contrast, once a muscle fibre was innervated by only one axon then that cartel would activate all the remaining AChR regions synchronously. If asynchrony of receptor activation leads to synapse elimination, then we hypothesized that it should be possible to perturb synaptic maintenance even at singly innervated junctions by desynchronizing AChR activation at different sites within a junction.

Changes induced by focal AChR blockade

AChR activation within a junction was desynchronized by focally blocking neuromuscular transmission with α-bungarotoxin (αbtx), an irreversible ligand of the AChR which prevents receptor activation by acetylcholine. This was accomplished in the sternomastoid muscle of living adult mice by applying many pulses of positive pressure to a micropipette filled with either fluorescently labelled or unlabelled αbtx in saline positioned over one region of a superficial neuromuscular junction under visual control (Fig. 1). In most experiments the same site was saturated with αbtx two times 48–72 h apart.

At many neuromuscular junctions, focal blockade of neuromuscular transmission elicited pre- and postsynaptic changes beginning several days after the application of αbtx. The principal change was a gradual and progressive elimination of synaptic sites that were blocked. Figure 2 shows an example of a junction in which about 20% of the AChRs throughout the junction were labelled with rhodamine conjugated αbtx (Rαbtx) on day 0 (a dose that is unlikely to interfere with effective neuromuscular transmission[13]) and the bottom region was then twice saturated with unlabelled αbtx (day 0 and day 3). Signs of AChR loss were evident three days after the second application of unlabelled αbtx (Fig. 2, bottom row, day 6). Over the subsequent 5 days, progressively more of the previously stained AChRs in the blocked area disappeared (Fig. 2, bottom row, days 6–11) showing that AChRs inserted before the blockade were lost from that region. At day 31, additional Rαbtx was bath applied to the junction to label AChRs inserted since the time of the first focal blockade (little of the original Rαbtx staining was left anywhere because of turnover of AChRs). Although the additional Rαbtx stained the top of the junction at day 31 no new staining was evident in the region that was previously blocked. Thus, AChR molecules already in the postsynaptic membrane selectively disappeared from blocked sites (by either migration away or internalization) faster than turnover caused receptors to be removed from unblocked parts of the junction. In addition, the blocked region of the junction either did not incorporate any newly synthesized AChRs into its membrane or did not maintain a detectable number of the AChRs that were incorporated there.

The other major change we observed at the sites of focal blockade was loss of presynaptic nerve terminal staining. Using 4-Di-2-Asp to stain motor nerve terminal mitochondria[1,2,11], we observed loss of brightly stained presynaptic elements from the area overlying the region of AChR blockade. The loss of nerve terminal staining induced by focal blockade progressed over the same interval as loss of AChR staining (Fig. 2, top row). The protracted nature of these changes suggests that focal blockade sets into motion a relatively slow process that continues until the blocked region is eliminated. The fact that no nerve terminal sprouts or AChRs reappeared at the previously blocked sites one month later suggests that the changes induced by focal blockade are permanent. Permanent loss of pre- and postsynaptic staining also occurs in other situations in which synapse elimination takes place[2,11,14,15].

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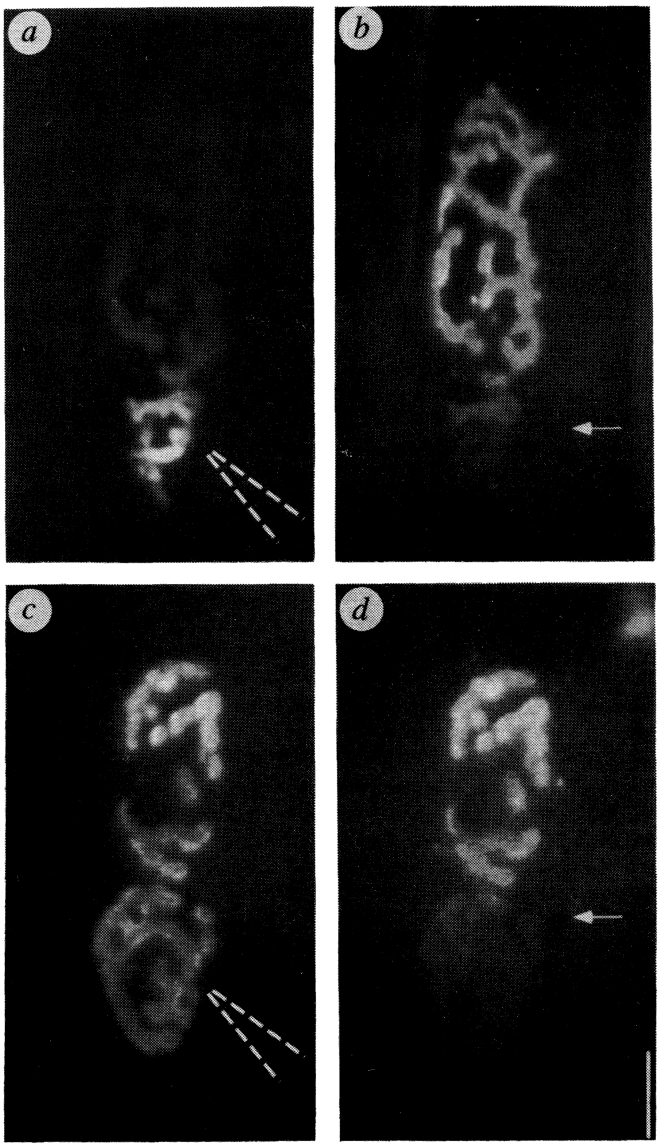

FIG. 1 Focal application of α-bungarotoxin to regions of adult neuromuscular junctions saturates AChRs. Adult mice (25–25 g) were anaesthetized and the sternomastoid muscle exposed as previously described[1]. *a*, A microelectrode, pulled to have a sharply tapering shank typical of an intracellular electrode with a blunt end typical of a patch electrode (tip diameter, <1 μm, resistance 7–10 mOhms measured in 3M KCl) was filled with a mixture of 20–25 μg ml^{-1} rhodamine conjugated αbtx (Rαbtx[1,2,11]), 25–50 μg ml^{-1} unlabelled αbtx (BioToxins, Inc.) and 10 μg ml^{-1} sulphorhodamine 101 (Molecular Probes) as a fluorescent tracer in lactated Ringer's (Baxter). The electrode (dashed lines) was positioned near a junction under epillumination using a polarizing filter cube and long working distance ×40 water immersion objective (Zeiss, 0.75 n.a.). Perfusion of Ringer's (10 ml min^{-1}) across the surface of the muscle was adjusted so that the stream from the electrode was directed toward the area of interest and away from the rest of the junction (see Fig. 2, bottom row, 'during puffing'). Small quantities of the αbtx solution were then released from the tip of the electrode using pressure pulses (10–20 p.s.i.) delivered at 5–20 Hz in trains of 50–500 ms duration for 5–10 min. *b*, In control experiments, the degree of saturation was determined by subsequently applying Lucifer yellow conjugated αbtx (LYαbtx, Molecular Probes) and noting the presence or absence of LYαbtx binding in the region of the junction near the electrode (region below arrow). Motor nerve terminals were subsequently stained with a vital, non-toxic mitochondrial dye, 4-Di-2-Asp[1,2,11] to determine whether or not the overlying motor nerve terminals were damaged during the application of αbtx (not shown but see Fig. 2). Digital images of neuromuscular junctions were obtained using a low-light SIT video camera and an image processor as described previously[1,2,11]. Two to three days later, the entire procedure was repeated. Junctions were then followed over the next 1 to 8 weeks at intervals ranging from 2 days to 2 weeks. *c*, To prevent the possibility of photodamage to Rαbtx saturated AChRs having artefactually induced synapse loss, in some experiments (10 junctions in 5 mice) focal blockade was accomplished with unlabelled αbtx. Neuromuscular junctions were briefly stained (1–2 min) with Rαbtx so that less than 20% of the postsynaptic AChRs were labelled. In this way the postsynaptic distribution of AChRs could be visualized while leaving synaptic transmission unaffected. A microelectrode (dashed lines) filled with unlabelled αbtx (100 μg ml^{-1}) in Ringer's solution was positioned near a junction and small quantities of αbtx released with pressure as described above. *d*, In control experiments, saturation was checked with a subsequent application of LYαbtx. The parameters, in particular the number of puffs, necessary for saturating a junctional area were determined from this kind of control experiment. In a few experimental junctions, a low dose of LYαbtx was applied after puffing, as in the control experiments, to confirm the blockade without saturating the rest of the junction. In all figures digital images of multiple focal planes were aligned and out of focus blur was removed using previously described computational methods[10]. Scale bar, 20 μm.

Of 55 junctions (in 23 mice) receiving focal αbtx to the same site twice, 19 (~35%) showed loss of pre- and postsynaptic staining comparable to that shown in Fig. 2. Thirteen (~24%) showed no evidence of pre- and postsynaptic elimination and 23 (~42%) showed changes that indicated they were inadvertently damaged by the procedure, resulting in denervation (absence of 4-Di-2-Asp staining) and/or muscle fibre degeneration (presence of cellular debris and absence of striations). Junctions which showed no evidence of loss had on average larger blocked areas than the junctions which did change (see below). Focal blockade was also less successful at inducing synaptic changes when junctions were blocked only once. Of 65 junctions in 30 mice in which αbtx was applied only one time, 12 (~18%) showed loss of pre- and postsynaptic staining, 22 (~34%) showed no evidence of pre- or postsynaptic change and 31 (~48%) were damaged by the procedure or not relocated.

Two observations indicated that the loss of presynaptic staining observed with the mitochondrial stain 4-Di-2-Asp was due to the frank withdrawal of motor nerve terminals from blocked sites rather than a redistribution of mitochondria within the nerve terminal. Use of a fluorescent dye that intercalates into nerve terminal membrane (RH 795; ref. 2) showed that regions that had lost 4-Di-2-Asp staining could no longer be stained with RH 795 (6/6 junctions in 3 mice).

In addition, a change in synaptic basal lamina staining indicated structural withdrawal of nerve terminals overlying blocked postsynaptic sites. Acetylcholinesterase and the lectin VVA[16] that stains it are distributed in a 'railroad-track' pattern at normal junctions and fills in to become a single broad band (presumably due to increased access) after nerve terminals have withdrawn[11,14,17]. At sites that were focally blocked and lost pre- and postsynaptic staining, VVA filled in (4/4 junctions from 2 mice; Fig. 3).

We studied the temporal relationship between nerve terminal withdrawal and AChR loss at sites of focal blockade. In eight junctions that showed evidence of changes 2–3 days after focal blockade, we continued to make observations at 2–3 day intervals during the process of synapse elimination. In each of these junctions, we found time points at which AChR staining was diminished in the blocked sites whereas nerve terminal staining was still present (Fig. 4). In none of these junctions did we see the opposite: nerve terminal staining missing at sites that had normal Rαbtx staining. At most blocked sites we observed either that nerve terminal staining and the AChR staining had already both been lost, or that both pre- and postsynaptic staining still appeared normal. From these results we conclude that nerve terminal withdrawal lags slightly the onset of AChR loss from blocked sites. Thus, the timing of pre- and postsynaptic loss

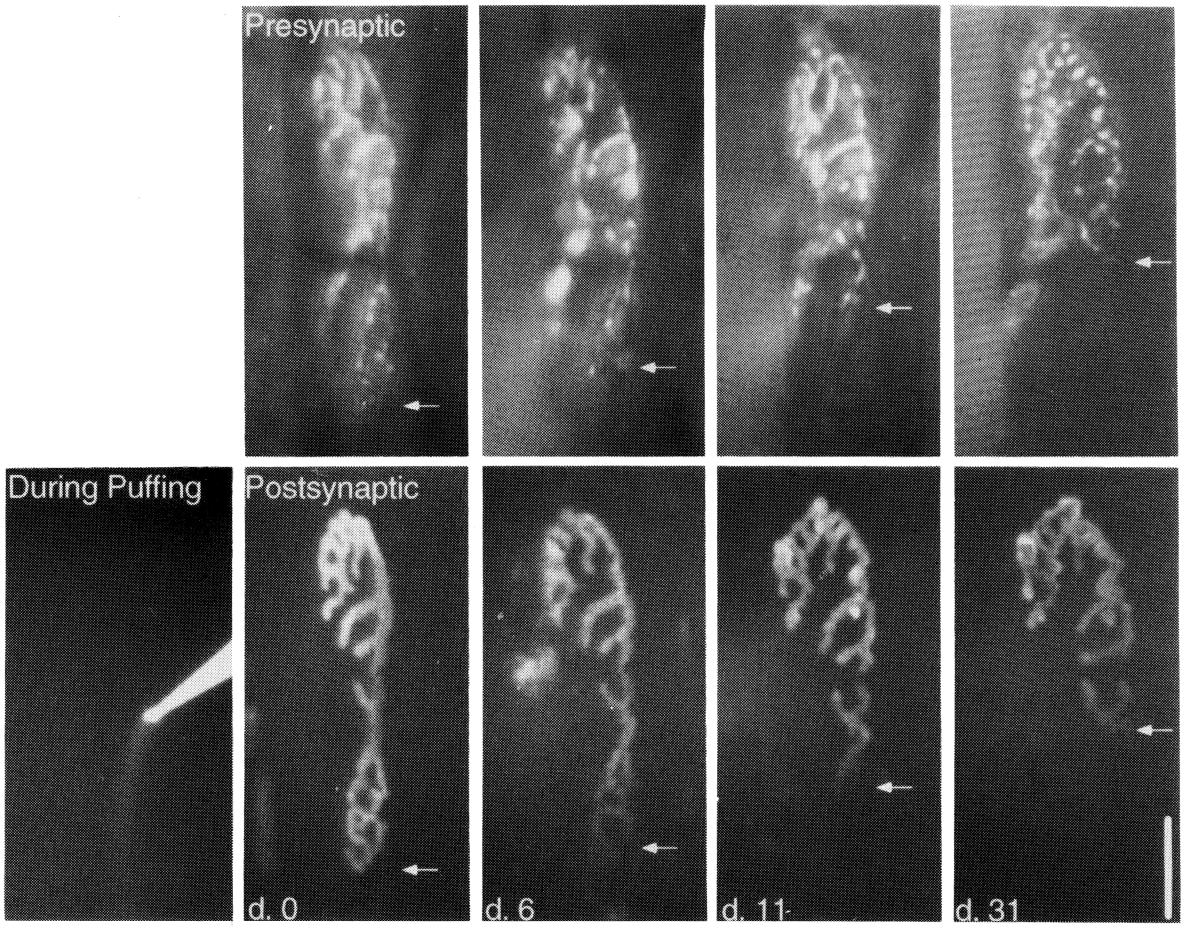

FIG. 2 Gradual loss of presynaptic motor nerve terminal and postsynaptic AChR staining from synaptic regions saturated with αbtx. Following staining of about 20% of the AChRs throughout the junction with Rαbtx and saturating all the AChRs in the botton region of this neuromuscular junction with unlabelled αbtx (panel labelled 'During Puffing'), presynaptic motor nerve terminals (top row) and postsynaptic AChRs already in the membrane (day 0–11, bottom row) were followed over time. Immediately following application of αbtx (day 0), motor nerve terminal staining was present over the synaptic regions containing saturated AChRs. Over the next several days to weeks, there was a progressive loss of motor nerve terminal staining as well as of the preexisting AChRs (arrows). Moreover, the loss of these areas appeared to be permanent because 31 days later no staining was observed in the previously saturated region of the junction, either using 4-Di-2-Asp (top) or when for the first time additional Rαbtx was applied (bottom). At the last view the remaining AChR-rich areas seem somewhat enlarged perhaps as compensation for the lost sites. The gradual and permanent nature of synaptic change induced by focal blockade of synaptic transmission in one region of an adult neuromuscular junction is similar to that observed during naturally occurring synapse elimination during development[2]. Scale bar, 20 μm. We did a variety of control experiments which ruled out the possibility that the changes observed were artefactual. We attempted to damage nerve terminals locally by touching a small region of the nerve terminal with a puffing pipette lacking αbtx (24 junctions in 7 mice). In 11 junctions no change was observed; in the 13 junctions in which changes were observed, these consisted of denervation and subsequent reinnervation of the entire junction (4 junctions); and in the remainder, denervation and muscle fibre degeneration and regeneration was observed (9 junctions). In an additional 6 junctions in 3 mice the same solution minus αbtx was focally applied twice according to the protocol described above without damaging the junction. In none of these cases did we observe motor nerve terminal or AChR loss, indicating that αbtx in the pipette was necessary to induce synaptic changes. Moreover, focal application of unlabelled αbtx was as effective as fluorescently tagged αbtx, indicating that local phototoxicity could not have caused elimination of the blocked sites.

induced by focal blockade is analogous to that which occurs during synaptic competition between different axons during development and reinnervation[2,11].

Blockade throughout a junction

To evaluate the possibility that the changes we observed were caused by the puffing technique itself rather than focal blockade of neurotransmission, we did a variety of control experiments each with the aim of inducing synaptic loss without focally blocking neuromuscular transmission. In none of these experiments could we replicate the effects of focal blockade (Fig. 2 legend).

Saturating the entire junction with two 30-min bath applications of rhodamine or unlabelled αbtx 48–72 h apart in the same solution used in the puffing pipette also caused no sign of pre- or postsynaptic loss in any of the junctions studied (22 junctions in 6 mice; Fig. 5). The inability of a complete blockade of neuromuscular transmission to cause synapse elimination indicated that αbtx itself was not causing synapse loss through, for example, an unexpected impurity that is toxic to synaptic sites. In addition, however, the inability of widespread junctional blockade to elicit synaptic loss implied that neuromuscular blockade is not itself sufficient to cause the changes we observed. Rather, these results suggest that inactive junctional regions are eliminated only in the presence of ongoing activity elsewhere in the junction.

To study this possibility further we examined 50 junctions in which the focal application of fluorescently labelled αbtx (to show the size of the blocked region) or a subsequent brief application of Lucifer yellow αbtx (to counterstain the blocked area) allowed us to measure the size of the region labelled by puffing. These partially blocked junctions were divided into two groups: those that showed loss and those that did not. The per cent of the junctional area blocked for each group is shown in Fig. 6.

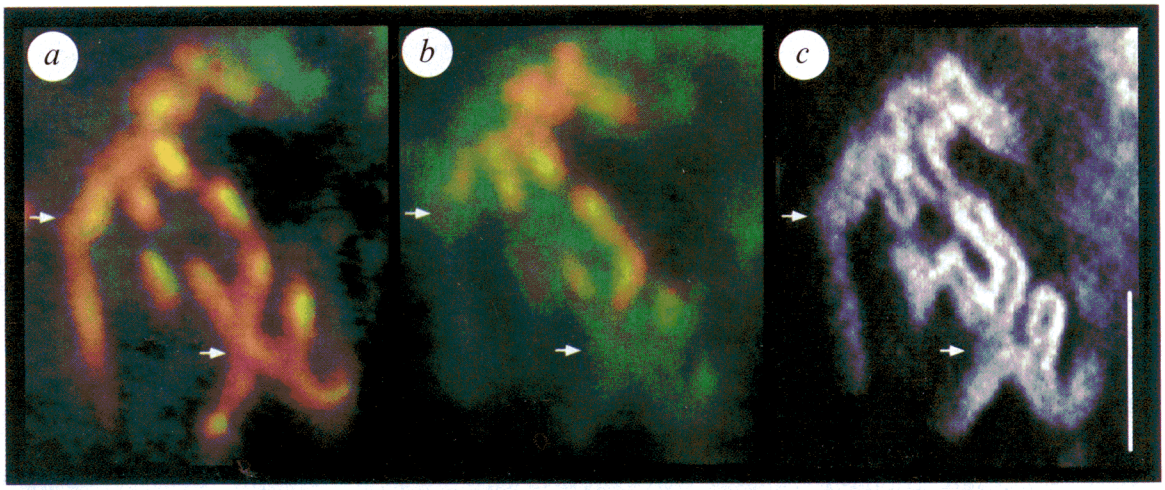

FIG. 3 Evidence that presynaptic motor nerve terminals withdraw from postsynaptic areas saturated with αbtx. *a*, Shown is a portion of a neuromuscular junction saturated twice with Rαbtx. Immediately following application of Rαbtx, motor nerve terminal staining with 4-Di-2-Asp (green) was observed over the region of the junction containing saturated AChRs (red). At sites of overlap the juxtaposition of green and red appears yellow. *b*, 21 days later, motor nerve terminals were again stained with 4-Di-2-Asp and AChRs were re-stained with Rαbtx (red). Former pre- and postsynaptic sites were no longer stained in the region that was previously blocked. *c*, At the time of the second view (same time point as in *b*), the synaptic basal lamina was stained with FITC-conjugated lectin, VVA, 10 μg ml^{-1}, ref. 16). The 'railroad-track' appearance of VVA staining is visible in regions of the junction in which motor nerve terminal staining is still present (compare areas above arrows in *b* and *c*), probably because the nerve terminal retards diffusion of the lectin into the synaptic cleft. Where no motor nerve terminal staining remains (areas below arrows in *b* and *c*), the VVA staining has been filled in, typical of areas that have lost motor nerve terminals[11]. Thus, loss of motor nerve terminal staining is due to frank structural withdrawal of motor nerve terminals from blocked regions of the junction. Scale bar, 20 μm.

Junctions in which synapse loss was observed had on average smaller blocked regions than junctions that showed no loss (Fig. 6*a*). The probability of loss decreased as the size of the blocked region increased (Fig. 6*b*) indicating that blocked sites were only lost when a substantial area of the junction remained unblocked.

In another series of experiments, we denervated the sternomastoid muscle to eliminate all neuromuscular transmission and then focally blocked regions of neuromuscular junctions (8 junctions in 3 mice). AChR loss was not observed in any of these junctions. Thus non-focal blockade, blockade of large postsynaptic regions and focal blockade in the absence of the nerve were all ineffective in causing synaptic changes. These results strongly suggest that ongoing activity in the remaining parts of the junction is necessary to induce elimination of the blocked regions.

Discussion

The experiments of Hubel and Weisel on the segregation of ocular dominance columns during early postnatal life underscored the importance of the relative activity of competing inputs in determining the final pattern of synaptic connections in the nervous system. Whereas patching one eye profoundly diminished the ability of the inactive eye to maintain synaptic connections in visual cortex, patching both eyes had a far less dramatic effect[18,19]. This work made a strong case for the idea that differences in neural activity between the eyes, rather than the overall amount of activity, determine the outcome of the competition between the two eyes for cortical space. These landmark experiments firmly established the relationship between neural activity and long-term synaptic modifications that occur in the nervous system as a consequence of experience.

The experiments we report here seem to show an analogous competitive process between effective and ineffective synapses on individual postsynaptic cells. Blocking activity at all synaptic sites within a neuromuscular junction has far less of an effect than blocking a portion of the sites. Thus the context within which a synaptic site is inactive is critically important to its fate. This work also shows that in certain circumstances synaptic connections in adult animals can undergo the kind of permanent changes that is the hallmark of activity-mediated plasticity during critical periods in development.

Ordinarily in adulthood, the stage at which these experiments were done, all the presynaptic terminals at a neuromuscular junction originate from the same axon and are thus a synchronously active cartel[12] whose terminals normally coexist stably for very long times[20–22]. If, however, an adult neuromuscular junction is innervated by more than one axon, as occurs following reinnervation[11] or following muscle fibre regeneration[23], then synapse elimination ensues until only one cartel remains. The experiments reported here suggest that if the activity patterns of each synaptic cartel multiply innervating a junction were different, then that difference alone might be sufficient to cause syn-

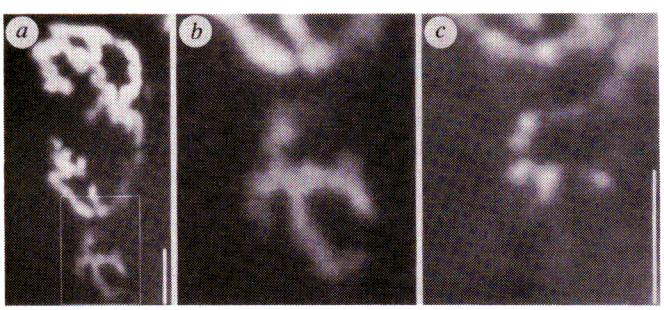

FIG. 4 Loss of postsynaptic AChRs occurs before withdrawal of overlying motor nerve terminals. *a*, Shown is a neuromuscular junction in which the bottom area was saturated 17 days earlier with Rαbtx. At this view, additional Rαbtx was bath applied to the muscle to reveal the remaining synaptic regions. The bottom region of this junction is in the process of being eliminated, as indicated by the decreased brightness of Rαbtx staining in the bottom and along the right branch in this area. *b*, Higher magnification view of the boxed region of junction shown in *a*. *c*, 4-Di-2-Asp staining of the motor nerve terminal in the region in *b* shows partial occupation of faintly stained AChRs. Some faintly staining AChR regions are still occupied by nerve terminals indicating a change in postsynaptic AChR density before nerve terminal withdrawal. Scale bar, 20 μm.

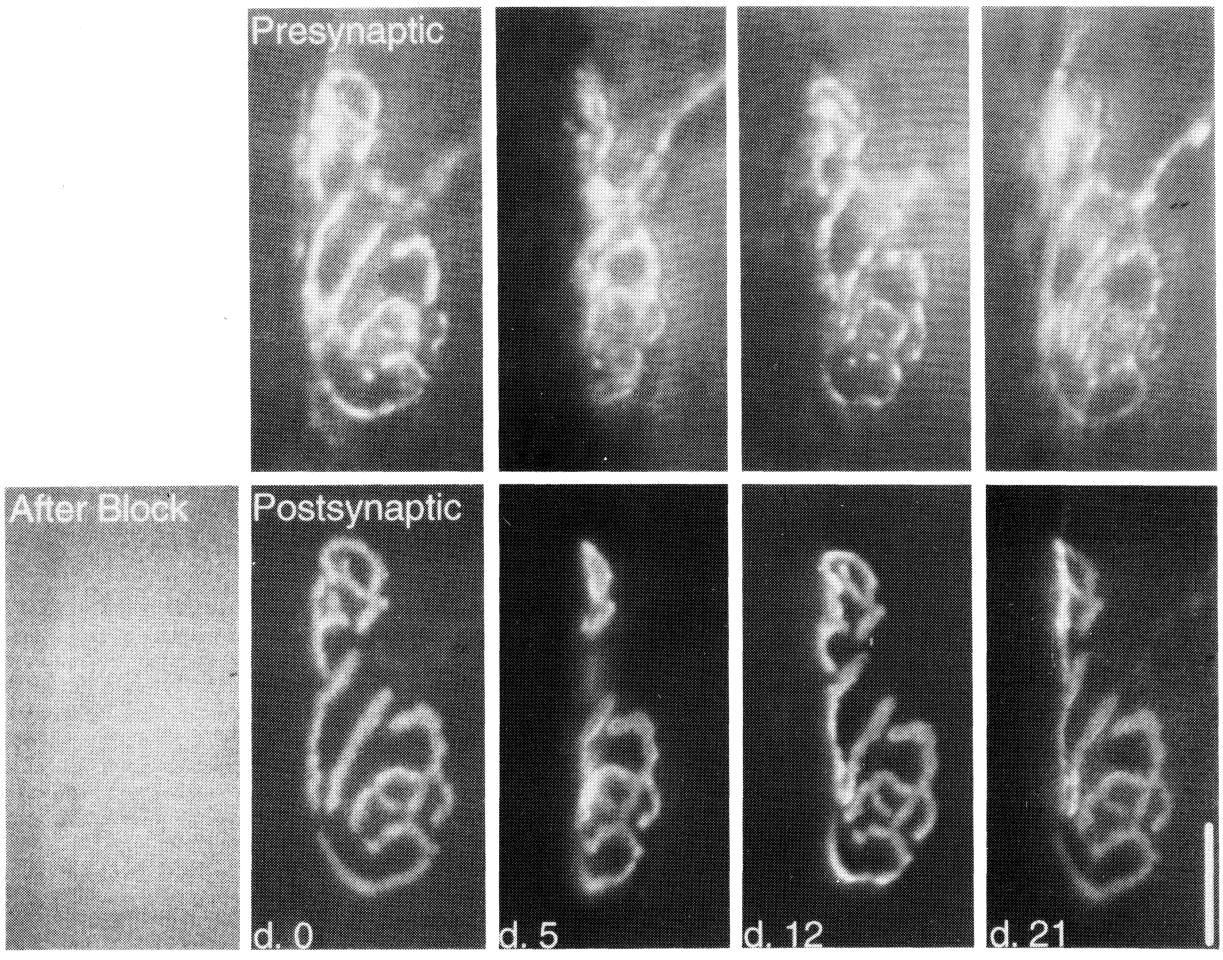

FIG. 5 Blocking postsynaptic AChRs throughout a junction does not result in loss of pre- or postsynaptic sites. Following labelling of about 10% of the postsynaptic AChRs with Rαbtx (day 0), unlabelled αbtx was applied to junctions until all of the remaining AChRs were saturated. Saturation was confirmed by subsequently applying LYαbtx to the junction; no staining was observed (panel labelled 'After Block'). AChRs were saturated again 2 days later (not shown). Presynaptic motor nerve terminals (top) and postsynaptic AChRs (bottom) were then followed over 21 days. No loss of pre- or postsynaptic sites was observed. The junction appears thinner at day 5 probably due to slight rotation of the muscle fibre at this view. These results shows that the blockade of AChRs with αbtx itself does not induce synaptic loss. Following complete blockade the percentage of blocked AChRs gradually declines because of the receptor turnover[45]. Because nerve terminal loss was not observed at any time following blockade, this experiment shows that there is no threshold level of blockade that causes synapse elimination. Thus, block of 100% of the AChRs in 30% (or less) of the junction (see Fig. 6) induces loss, whereas blocking 30% of the AChRs in 100% of the junction is ineffective. Thus, asymmetry in the efficacy of neuromuscular transmission among the different synaptic sites within a neuromuscular junction is necessary to induce loss. Scale bar, 20 μm.

apse elimination. In adult animals, in fact, motor axons projecting to the same muscle typically are not synchronously active but are recruited in a fixed order[24,25]. One consequence of this is that on multiply (but not on singly) innervated muscle fibres, some sites will be active while others are inactive.

The role of activity in synapse elimination at the neuromuscular junction is complex and controversial. Although some experiments suggest that active axons have a competitive advantage over inactive axons[26], others support the opposite conclusion[27] and many other experiments suggest a modulating role of activity on the rate of the elimination process (see for example refs 28–31). The present experiments show that a small inactive site is eliminated when pitted against a large active site, but that a smaller active site is neither eliminated by, nor able to eliminate an inactivated region. This argues that in muscle, first, only a threshold amount of postsynaptic activation can destabilize inactive sites and second that an inactive postsynaptic cell may not be able to cause weakening of an active synapse (but see ref. 32).

Previous studies raised the possibility of an important mediating role for the muscle fibre in synapse elimination because decreases in the density of postsynaptic AChRs began before loss of presynaptic terminals[2,11]. The same sequence was seen in the present experiments. Although these results suggest that changes in a postsynaptic site could initiate presynaptic terminal withdrawal, they are also consistent with the possibility that nerve terminals instigate their own demise by causing local AChR disappearance and their own disassembly. The present experiments, however, show that a change in the postsynaptic cell (focal blockade) is sufficient to cause normal innervating nerve terminals to withdraw. This strongly supports the idea that the postsynaptic cell plays an important part in maintaining, or choosing not to maintain, the synaptic connections impinging on it implicating retrograde signals in the synapse elimination process. Activity-mediated signals emanating from muscle fibres may also affect pre-synaptic efficacy[33,34].

The molecular cascade that underlies synapse elimination is unknown. Local differences in the timing of activation of postsynaptic AChRs presumably lead to local differences in the stability of receptors in the postsynaptic membrane. Receptor disappearance could occur because the AChRs migrate away. The 43K protein that anchors AChRs in the postsynaptic membrane[35,36] disappears from sites undergoing synapse elimination at the same rate as AChRs themselves[37]. AChRs could

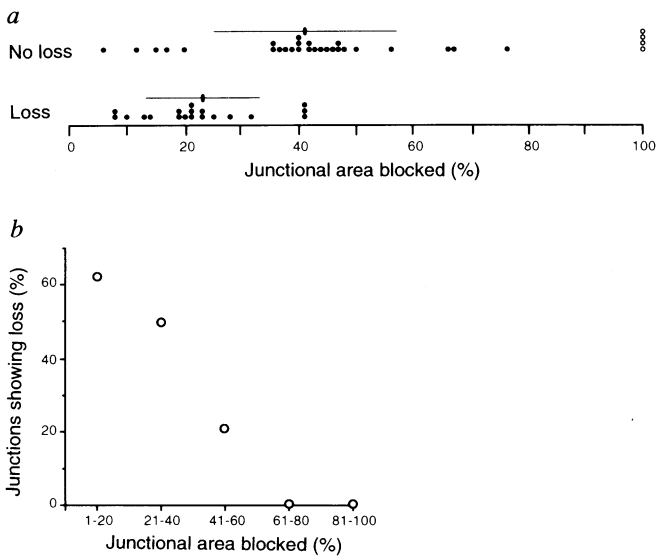

FIG. 6 Probability of synaptic loss is related to the percentage of junctional area focally blocked. *a*, Fifty focally blocked junctions (single and double application) in which we were able to assay the area of the blocked region were separated into two groups, those which did not undergo synaptic loss (No Loss) and those which did (Loss). Although the receptor labelling was usually in a gradient, we could in each junction discern a region of similar intensity (the area of saturation). The percentage of junctional area which was saturated was plotted for each junction (filled circles). Four junctions in which the entire junctional area was saturated are indicated with unfilled circles (these junctions were not included in the analysis of the mean). The mean (vertical line) and standard deviation (horizontal line) are indicated for each group. Of junctions in which 30% or less of the junctional area was focally blocked, 15 of 20 underwent synapse elimination. In contrast, of junctions in which 30% or more of the junctional area was blocked, only 4 of 30 showed evidence of loss. None of the junctions in which greater than 41% of the junctional area was blocked underwent synapse loss. *b*, The percentage of junctions which underwent synapse elimination (circles) is plotted against the per cent of junctional area focally blocked. As the percentage of blocked junctional area increases, the probability of loss decreases. These data indicate that inactive synaptic regions are at risk for elimination only when some threshold level of activation remains in the rest of the junction.

also migrate by virtue of electrophoresis of membrane proteins as a consequence of voltage gradients induced by activity[38–40]. Alternatively, a change in the availability of nerve-derived AChR clustering or maintenance factors[41,42] or their receptors[43,44] may cause the local disappearance of AChRs within a junction.

AChR clusters could also disappear if the internalization of AChRs is accelerated at inactive sites. Increased turnover (removal and addition) of AChRs occurs in muscles that are inactive[45]. However, inactivity itself does not cause postsynaptic sites to disappear (Fig. 5, see also ref. 11). Rather, AChR activation at one site would have to initiate selective internalization of AChRs at nearby inactive sites. Such a view was essentially posited by Stent in a theoretical treatment of Hebbian mechanisms underlying activity-dependent synaptic plasticity[46]. He proposed that postsynaptic action potentials could cause the internalization of inactive receptors. Consistent with this, tonic muscle fibres which do not fire action potentials do not undergo synapse elimination[47].

It seems unlikely that the activity of each individual AChR determines whether it is maintained or eliminated. In the blocked region all AChRs disappear including any unblocked AChRs (such as those inserted after the blockade is completed). Similarly with a low dose of αbtx some fraction of AChRs are blocked outside the focally blocked area and these AChRs persist (see for example Fig. 2). The results thus suggest that the ensemble activity of a postsynaptic region is the critical factor that determines its fate. Activity-dependent accumulation of a chemical signal (such as calcium) or production of an electrical membrane signal (such as voltage) may, if it reaches some threshold, protect any receptors within an active region whether they themselves are active or not. This may explain why despite the gradient of blockade produced by αbtx application there was utlimately a sharp boundary between the lost and maintained regions.

In addition to AChR loss, focal postsynaptic blockade also causes the permanent withdrawal of the overlying nerve terminal. Focal blockade could cause synapse loss by restricting trophic feedback from the postsynaptic cell so that some terminals are nourished as adjacent terminals are deprived. Alternatively, it may only be necessary that the adhesive bonds that affix a nerve terminal to the basal lamina overlying postsynaptic sites are eliminated within the focally blocked area. A role for adhesion during periods of muscle growth[20] and atrophy[21] suggests that the adhesive bonds between pre- and postsynaptic elements may be critical to nerve terminal maintenance. Proteases have been implicated in the elimination process[48,49] and could potentially break these adhesive bonds. The ability to predict exactly where synapse elimination is going to occur using the focal blockade approach should allow a careful analysis of these possibilities. □

Received 26 July; accepted 26 October 1994.

1. Lichtman, J. W., Magrassi, L. & Purves, D. *J. Neurosci.* **7,** 1215–1222 (1987).
2. Balice-Gordon, R. J. & Lichtman, J. W. *J. Neurosci.* **13,** 834–855 (1993).
3. Wigston, D. J. *J. Neurosci.* **9,** 639–647 (1989).
4. Langenfeld-Oster, F., Dorlochter, M. & Wernig, A. *J. Neurocytol.* **22,** 517–530 (1993).
5. Hill, R. R., Robbins, N. J. & Fang, Z. P. *J. Neurocytol.* **20,** 165–182 (1991).
6. Herrera, A. A., Banner, L. R. & Nagaya, N. *J. Neurocytol.* **19,** 85–99 (1990).
7. Chen, L. L., Folsom, D. B. & Ko, C.-P. *J. Neurosci.* **11,** 2920–2930 (1991).
8. Redfern, P. A. *J. Physiol.* **209,** 701–709 (1970).
9. Brown, M. C., Jansen, J. K. S. & Van Essen, D. *J. Physiol.* **261,** 387–422 (1976).
10. Balice-Gordon, R. J., Chua, C. K., Nelson, C. C. &. Lichtman, J. W. *Neuron* **11,** 801–815 (1993).
11. Rich, M. M. & Lichtman, J. W. *J. Neurosci.* **9,** 1781–1805 (1989).
12. Colman, H. & Lichtman, J. W. *Devl Biol.* **156,** 1–10 (1993).
13. Lingle, C. & Steinbach, J. H. *Int. Anesth. Clin.* **26,** 288–301 (1988).
14. Rich, M. M. & Lichtman, J. W. *Neuron* **3,** 677–688 (1989).
15. Rich, M. M., Colman, H. & Lichtman, J. W. *Neurology* **44,** 2138–2145 (1994).
16. Scott, L. J. C., Bacou, F. & Sanes, J. R. *J. Neurosci.* **10,** 346–350 (1990).
17. Wernig, A. M., Pécot-Dechavassine, M. & Stöver, H. *J. Neurocytol.* **9,** 277–303 (1980).
18. Hubel, D. H. & Wiesel, T. N. *J. Neurophysiol.* **28,** 1041–1059 (1965).
19. Wiesel, D. H. & Hubel, T. N. *J. Physiol.* **28,** 1029–1040 (1965).
20. Balice-Gordon, R. J. & Lichtman, J. W. *J. Neurosci.* **10,** 894–908 (1990).
21. Balice-Gordon, R. J., Breedlove, S. M., Bernstein, S. & Lichtman, J. W. *J. Neurosci.* **10,** 2660–2671 (1990).
22. Wigston, D. J. *J. Neurosci.* **10,** 1753–1761 (1990).
23. van Mier, P. & Lichtman, J. W. *J. Neurosci.* **14,** 5672–5686 (1994).
24. Henneman, E. *Science* **126,** 1345–1347 (1957).
25. Burke, R. E., in *Myology*. (eds Engel, A. G., Franzini-Armstrong, C.) 464–484 (McGraw Hill, New York, 1994).
26. Ridge, R. M. A. P. & Betz, W. J. *J. Neurosci.* **4,** 2614–2620 (1984).
27. Callaway, E. M., Soha, J. M. & Van Essen, D. C. *Nature* **328,** 422–426 (1987).
28. Thompson, W. *Nature* **302,** 614–616 (1983).
29. Srihari, T. & Vrbová, G. *J. Neurocytol.* **7,** 529–540 (1978).
30. Ribchester, R. R. *J. Physiol.* **40,** 53–75 (1988).
31. Benoit, P. & Changeux, J.-P. *Brain Res.* **149,** 89–96 (1978).
32. Hata, Y. & Stryker, M. P. *Science* **265,** 1732–1735 (1994).
33. Lo, Y. J. & Poo, M.-M. *Science* **254,** 1019–1022 (1991).
34. Dan, Y. & Poo, M.-M. *Science* **256,** 1570–1573 (1992).
35. Phillips, W. D. *et al. Science* **251,** 568–570 (1991).
36. Froehner, S. C., Luetje, C. W., Scotland, P. B. & Patrick, J. *Neuron* **5,** 403–410 (1990).
37. Culican, S. M., Cohen, J. B. & Lichtman, J. W. *Soc. Neurosci. Abstracts* **19,** 1294 (1993).
38. Fraser, S. & Poo, M.-M. *Curr. Top. dev. Biol.* **17,** 77–100 (1982).
39. Poo, M.-M. *A. Rev. Neurosci.* **8,** 369–406 (1985).
40. Luther, P. W. & Peng, H. B. *J. Cell Biol.* **100,** 235–244 (1985).
41. McMahan, U. J. *Cold Spring Harbor Symp. quant. Biol.* **55,** 407–418 (1990).
42. Falls, D. L., Rosen, K. M., Corfas, G., Lane, W. S. & Fischbach, G. D. *Cell* **72,** 801–815 (1993).
43. Ma, J., Nastuk, M. A., McKechnie, B. A. & Fallon, J. R. *J. biol. Chem.* **268,** 25108–25117 (1993).
44. Corfas, G., Falls, D. L. & Fischbach, G. D. *Proc. natn. Acad. Sci. U.S.A.* **90,** 1624–1628 (1993).
45. Levitt, T. A., Loring, R. H. & Salpeter, M. M. *Science* **210,** 550–551 (1980).
46. Stent, G. S. *Proc. natn. Acad. Sci. U.S.A.* **70,** 997–1001 (1973).
47. Lichtman, J. W., Wilkinson, R. S. & Rich, M. M. *Nature* **314,** 357–359 (1985).
48. O'Brien, R. A. D., Ostberg, A. J. C. & Vbrová, G. *Neuroscience* **12,** 637–646 (1984).
49. Liu, Y., Fields, R. D., Fitzgerald, S., Festoff, B. W. & Nelson, P. G. *Proc. natn. Acad. Sci. U.S.A.* **91,** 10300–10304 (1994).

ACKNOWLEDGEMENTS. We thank H. Colman for helpful discussions. This work was supported by grants from the NIH and Muscular Dystrophy Association.