Rapid and Reversible Effects of Activity on Acetylcholine Receptor Density at the Neuromuscular Junction in Vivo

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Quantitative fluorescence imaging was used to study the regulation of acetylcholine receptor (AChR) number and density at neuromuscular junctions in living adult mice. At fully functional synapses, AChRs have a half-life of about 14 days. However, 2 hours after neurotransmission was blocked, the half-life of the AChRs was now less than a day; the rate was 25 times faster than before. Most of the lost receptors were not quickly replaced. Direct muscle stimulation or restoration of synaptic transmission inhibited this process. AChRs that were removed from nonfunctional synapses resided for hours in the perijunctional membrane before being locally internalized. Dispersed AChRs could also reaggregate at the junction once neurotransmission was restored. The rapid and reversible alterations in AChR density at the neuromuscular junction in vivo parallel changes thought to occur in the central nervous system at synapses undergoing potentiation and depression.

The efficacy of communication at synapses depends on the mechanisms that regulate the density of postsynaptic neurotransmitter receptors at sites of neurotransmitter release. The neuromuscular junction is a good site for studying the regulation of receptor density because of the unusually large number of acetylcholine receptors (AChRs) (*1*); the availability of α-bungarotoxin (α-bgt), an irreversible ligand, to label and thus quantify the number and density of AChRs (*2, 3*); and this synapse's accessibility, which allows in vivo study of the pre- and postsynaptic specializations over minutes or months (*4*).

AChR loss after a single saturating dose of bungarotoxin. We have taken advantage of the favorable features of the neuromuscular junction to study neurotransmitter receptor regulation in living animals, using a quantitative fluorescence assay to monitor changes in receptor number seen in multiple views of the same synapse (*3*). In the first series of experiments, the sternomastoid muscle in 70 mice was saturated with a single dose of tetramethyl-rhodamine–labeled α-bungarotoxin (r-α-bgt), and the fluorescence intensity of 220 neuromuscular junctions was assayed two or more times from 2 hours to 7 days later (Fig. 1A). Two hours after labeling, fluorescence intensity of the labeled receptors had decreased substantially 7.6 ± 2.3% [half-life ($t_{1/2}$) = 17.5 hours] (n = 50), and subsequent imaging of the same sites showed that the intensity continued to decrease over many days (Fig. 1B). Fluorescently labeled neuromuscular junctions in previously fixed muscles, however, did not lose intensity when imaged the same way, implying that this loss was a physiological process (*5*).

The rate of receptor loss (Fig. 1) was fast initially (~4% of label lost per hour at 2 to 4 hours after labeling, $t_{1/2}$ = ~17 hours) but slower later on (0.13% of remaining label lost per hour between 3 and 6 days, $t_{1/2}$ = ~13 days). Although this change in the rate of loss over time could be due to the coexistence of two populations of AChRs with different turnover rates (*6*), our results described below suggest that there is one population of receptors whose loss rate is dependent on the degree of neuromuscular blockade.

Rapid AChR loss during blockade of neuromuscular transmission. When we monitored receptor loss at junctions in which neurotransmission was chronically blocked with either curare or α-bgt (*7*), loss of receptors continued at a high rate of 4 to 5% per hour for at least 7 to 9 hours ($t_{1/2}$ = 13 hours) (Fig. 2A). This sustained high rate of loss contrasts with a decrease in the rate of loss beginning several hours after a single saturating dose (at ~7 hours, 65.6 ± 5.7% remained after chronic blockade; 83.2 ± 3.5% remained after a single saturating dose; $P < 0.0001$). When the chronic blockade experiment was accomplished with r-α-bgt rather than curare or unlabeled α-bgt (*8*), junctions (n = 10) still lost fluorescence intensity at an accelerated rate of 4 to 5% per hour. Thus, rapid loss of fluorescence in chronically blocked neuromuscular junctions in the previous experiment was not explained by displacement of r-α-bgt from AChRs by unlabeled bgt or curare. This result also indicates that the increased loss of receptors after neuromuscular

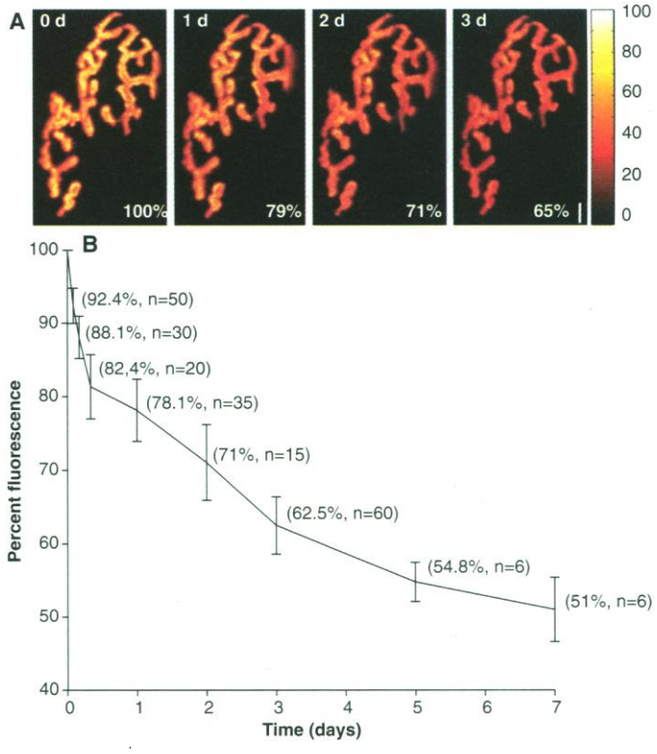

Fig. 1. Neuromuscular AChR loss after a single saturating dose of fluorescently labeled α-bgt in living mice. (**A**) Labeled AChRs at one neuromuscular junction were measured four times over 3 days (24 hours between views). The total fluorescence intensity (a measure of the total number of AChRs) was expressed as 100% at the time of saturation and determined on each successive view by comparing it with the fluorescence intensity of a nonbleaching standard (*3*). Pseudocolor images provided a linear representation of the density of AChRs (white-yellow, high density; red-black, low density). Scale bar, 10 μm. (**B**) Summary of data from all junctions with the approach shown in (A). Each data point represents the mean percentage of fluorescence intensity (±SD, n = number of junctions) at each view.

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blockade was not compensated for by an equivalent addition of new AChRs into the neuromuscular junction. As fluorescence intensity was lost, the junctions did not change in area (Fig. 2B), indicating that the synapse begins to reduce its receptor density within several hours of blockade of synaptic transmission. Our experiments could not determine at what rate receptors were inserted during chronic blockade but indicate that fewer receptors were being added than were being lost.

Slow AChR loss with nonblocking doses of α-bgt. In contrast, when relatively few AChRs (20 to 40%) were fluorescently labeled and thus neuromuscular transmission was still functional (*9*), the loss of label over 3 days was only ~14%, which corresponds to a mean loss rate of 0.19% per hour ± 0.07% (n = 38) (Fig. 2C). Indeed, at junctions that were not completely blocked, the loss of receptors over several hours was too small to detect with our methods. This low rate of loss was maintained for weeks (equivalent to an average half-life of ~14 days) (*10*). However, when junctions that were losing labeled receptors at a low rate were subsequently saturated with α-bgt, the rate of loss increased (Fig. 2D). The loss rate was unrelated to whether one or two α-bgt molecules were bound to each receptor (*11*). Thus, neuromuscular blockade rather than receptor occupation by blocking ligands induced accelerated receptor loss from the neuromuscular junction. Furthermore, a combination of saturating doses of curare and saturating doses of bgt did not have any additive effect on the rate of receptor loss, strongly suggesting that these drugs affected receptor loss by their action as receptor blockers.

In denervated muscles, neuromuscular blocking agents had no effect on the already accelerated AChR loss. In junctions that were denervated 7 to 10 days before, we found that AChRs were lost at an accelerated rate (~1% per hour, $t_{1/2}$ = 2.7 days) consistent with previous studies (*12*). The loss rate at denervated junctions was the same in the presence of either low or high doses of α-bgt (in both cases, ~1% per hour ± 0.01, n = 15). This result suggests that the activity of the muscle fiber rather than the number of AChRs bound by blocking agents is the variable that determines the loss rate.

Muscle activity prevents receptor loss. We next tested whether direct muscle stimulation would prevent the effects of blockade. Muscle fibers were saturated with r-α-bgt for 2 to 4 hours while being stimulated with extracellular electrodes (3-ms bipolar pulses of 6 to 9 V at 10 Hz for 1-s duration every 2 s). The electrodes were placed on the ends of the muscle so that direct muscle stimulation could occur without nerve activation. The result was dramatic: No evidence for receptor loss was observed in bgt-saturated muscles in the presence of directly elicited muscle twitching (Fig. 2E) (*13*). Even when receptor loss had already been accelerated by neuromuscular blockade with curare or saturating doses of α-bgt, the loss rate could be rapidly (within 2 hours) slowed down by stimulation (*14*). In animals that received a paralytic dose of tetrodotoxin (TTX) (to block voltage-gated Na^+ channels), but a nonblocking dose of r-α-bgt, receptor loss was not accelerated over the first few hours of TTX treatment. The inability of action-potential blockade to mimic the effects of synaptic blockade may be used to argue that inactivation of neuromuscular transmission is necessary for acceleration of receptor loss from junctions.

Receptor diffusion and internalization in the perijunctional region. We next studied the fate of AChRs that were lost from blocked neuromuscular junctions. After a single saturating dose of r-α-bgt followed by a saturating dose of curare for 5 to 8 hours, perijunctional rhodamine staining appeared (*15*), suggesting that receptors that are lost from blocked neuromuscular junctions migrate into the perisynaptic zone. The same result was found if unlabeled muscles were treated with a saturating dose of curare for 5 to 8 hours, and then labeled with r-α-bgt. In muscles where synaptic transmission was blocked for six or more hours, faint membrane staining was evident in the vicinity (50 to 100 μm) of the neuromuscular junctions, whereas in muscles viewed immediately after r-α-bgt application or 6 hours after a nonblocking low dose, there was little discernible perijunctional labeling (Fig. 3, A and B). The perijunctional staining appeared as multiple, very small (<1 μm) faint clusters. Nearest to the high-density AChR regions in the postsynaptic gutters, we sometimes saw what appeared to be streams of receptors that became less intense progressively with distance.

The AChRs that migrated into the perijunctional membrane were eventually internalized.

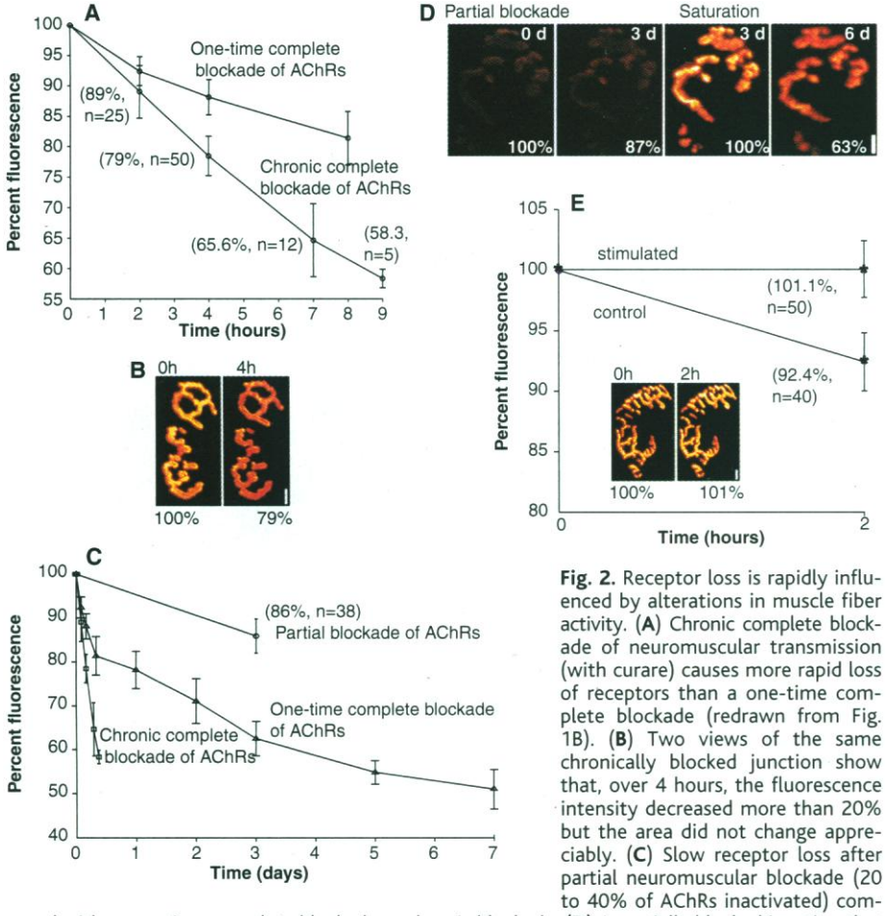

Fig. 2. Receptor loss is rapidly influenced by alterations in muscle fiber activity. (**A**) Chronic complete blockade of neuromuscular transmission (with curare) causes more rapid loss of receptors than a one-time complete blockade (redrawn from Fig. 1B). (**B**) Two views of the same chronically blocked junction show that, over 4 hours, the fluorescence intensity decreased more than 20% but the area did not change appreciably. (**C**) Slow receptor loss after partial neuromuscular blockade (20 to 40% of AChRs inactivated) compared with a one-time complete blockade or chronic blockade. (**D**) A partially blocked junction that lost receptor labeling at a low rate (13% in 3 days) could be made to lose AChRs at a high rate after being saturated with labeled α-bgt (37% loss in 3 days). The actual intensity of this junction at each view was well within the linear range of the silicon-intensified–target camera at moderate gain settings (*3*). All four panels are displayed on the same intensity scale. (**E**) Direct muscle stimulation for 2 hours prevented receptor loss from α-bgt-saturated neuromuscular junctions, compared with an 8% loss of receptor labeling without stimulation (inset shows two views of one blocked junction in a directly stimulated muscle). Error bars are standard deviations of the mean. Scale bars, 10 μm.

Time-lapse imaging of previously labeled AChRs after 8 hours of blockade with curare showed the appearance of small bright spots of fluorescence in the perijunctional region (Fig. 3, C and D). Imaging junctions from the side indicated that typically these spots were within the cytoplasm of the muscle fiber beneath the plasmalemma. The incidence of these spots was dependent on chronic blockade, because muscle fibers that received a single saturating dose of r-α-bgt showed many fewer spots than chronically blocked muscle fibers at 8 hours (mean of 0.63 ± 0.24 bright spots per junction after a single saturating dose, 4.13 ± 2.94 bright spots per junction after chronic blockade; $P < 0.00002$; Fig. 3, D and F). The perijunctional location of these spots suggested that they were formed by the internalization of perijunctional receptors. Indeed, the submembranous spots often first became visible in the perijunctional area several micrometers from the junction folds. We did not detect any net movement to or away from the junction (Fig. 3J). Other evidence also suggested that these spots were internalized AChRs. In denervated muscles known to be undergoing rapid receptor internalization, similar spots were seen. In particular, in muscle denervated 12 days earlier, spots also first appeared 8 hours after application of r-α-bgt (Fig. 3, H and I). In these denervated muscles, however, spots were found along the entire length of the muscle with an increase in density of about 30-fold in the perijunctional region. This distribution is consistent with the distribution of extrajunctional AChRs over the entire plasmalemma of denervated muscle fibers with a concentration of AChRs remaining at neuromuscular junctions (*16*).

Muscle activity induces reaccumulation of dispersed receptors. The fact that receptors spread into the perijunctional region from neuromuscular junctions during synaptic blockade raises the possibility that they might contribute to junctional receptor density once activity is restored. To test this idea, we lightly labeled junctional receptors (20 to 35%) with r-α-bgt, quantified the junction receptor number, and then blocked the remaining sites with curare. As expected, the labeled junctional receptor number decreased over 4 hours in the presence of blockade. At this point, the curare was washed out continuously with physiological saline to restore synaptic transmission. Four hours later, the junctions were reexamined without additional labeling. In each case ($n = 15$), the junctions had not only ceased to lose receptor number but in all cases the intensity had actually increased (Fig. 4, A and B). Thus, some receptors that are lost from inactive synapses can be reacquired when synaptic activity resumes, which suggests that the perijunctional receptor pool is both a source and a sink for junctional receptors.

Discussion. Although it has long been known that receptors in inactive muscle fibers undergo a transition over several days from long-lived to rapidly turning over [for review, see (*17*)], our results show that neuromuscular blockade and muscle stimulation can promote changes in AChR density and do so over shorter time frames. Changes in AChR density also occur during naturally occurring synapse elimination and can be experimentally induced by locally altering postsynaptic activity at part of a neuromuscular junction before synapses withdraw (*4*). It is possible that the phenomenon of synapse elimination and the rapid AChR loss described here use the same mechanism.

Our results suggest a model for the maintenance of the postsynaptic receptor density at the neuromuscular junction with four compartments: junctional receptors (J), perijunctional receptors (P), an internal pool of new receptors (I_{new}), and a pool of internalized receptors undergoing degradation (I_{deg}) (Fig. 4C). The existence of a perijunctional pool of receptors was previously suggested by virtue of the gradual decline in receptor density in the region surrounding the neuromuscular junction (*18*). Our results support the suggestion of Salpeter and Loring (*19*) that the perijunctional region acts as an "overflow zone" and is the site of receptor degradation once receptors are released from anchoring sites at the junction and migrate to the perijunctional region. Our work suggests a role for activity in regulating the equilibrium. In the absence of activity, the trend is for movement of receptors from the junction to the perijunctional region and thence into the internalized pool. With neuromuscular activity, the trend is in the opposite direction: Perijunctional receptors return to the junction and new receptors are also added. We do not know whether newly synthesized receptors are added directly to the junction or are inserted into the perijunctional region and then migrate into the junction to be anchored. That perijunctional receptors are the source of receptors for the junction is also supported by experiments with fluorescence recovery after photobleaching (*20*), suggesting that, even under normal conditions, the high density of receptors at the neuromuscular junction is in equilibrium with a pool of perijunctional receptors.

Neuromuscular blockade produced a rapid AChR loss, whereas action-potential blockade could not. It is possible that the basal amount of synaptic transmission that occurs during the spontaneous release of neurotransmitter must be prevented for receptor loss rate to increase. A continuing low level of receptor activation may also explain why AChR loss accelerates only after a substantial delay after muscle denervation (*21*). At denervated neuromuscular junctions, spontaneous release of neurotransmitter persists for days because of Schwann cell release of ACh packets (*22*). Analogously, only a basal level of synaptic transmission may be required to maintain postsynaptic structures in the CNS (*23*).

The way in which neuromuscular transmission or action potentials regulate AChR lifetime is not understood. The effects of inactivity on new receptor synthesis and denervation supersensitivity are mediated by Ca^{2+} influx (*24*). It is possible that a

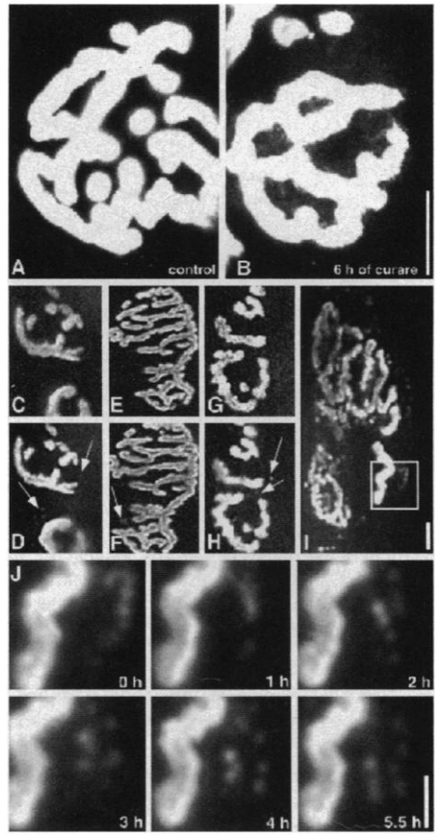

Fig. 3. Evidence for diffusion and internalization of AChRs from blocked neuromuscular junctions. (**A**) Neuromuscular junction saturated with tetramethyl-rhodamine–labeled α-bgt and viewed at high detector gain (the junctional branches are saturated) shows little evidence of perijunctional AChR labeling. (**B**) In contrast, a neuromuscular junction treated with a saturating dose of curare for 6 hours before labeling with r-α-bgt shows perijunctional rhodamine fluorescence. (**C** to **H**) Internalized receptor labeling is visible 8 hours after surface AChR labeling. Junctions viewed immediately after labeling with r-α-bgt (C, E, and G) show no internalized fluorescent spots in the perijunctional region, whereas 8 hours later (D, F, and H), internalized fluorescence spots appear. The number of spots is greater 8 hours after chronic curare treatment (D), or in muscles that were denervated 12 days earlier (H) than in controls (F) that were labeled with a one-time blockade dose of α-bgt. (**I**) Twelve days after muscle fiber denervation and 2 days after r-α-bgt labeling, large numbers of internalized spots are visible in the perijunctional region. (**J**) Internalized spots of fluorescence from region of inset in (I). The position and number of spots change over several hours. Scale bars, 10 μm. Arrows point to spots.

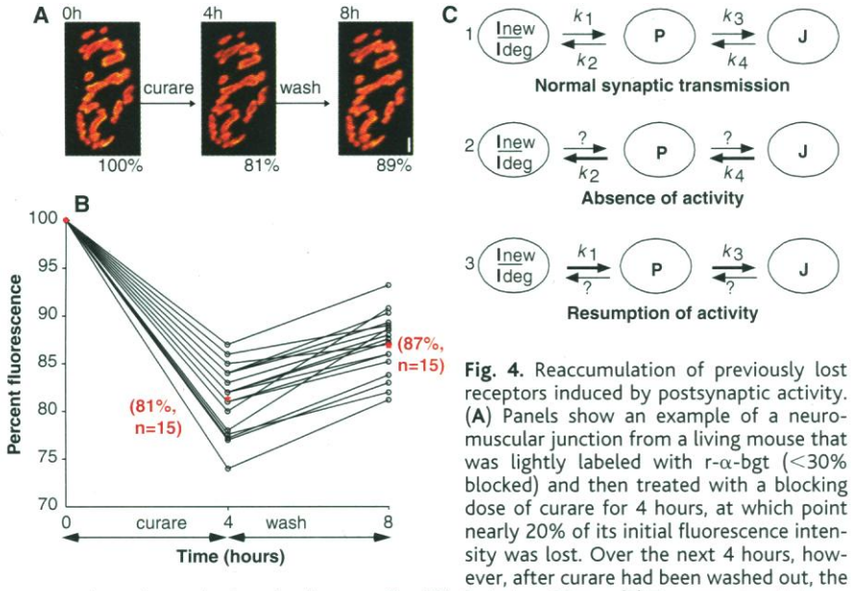

Fig. 4. Reaccumulation of previously lost receptors induced by postsynaptic activity. (**A**) Panels show an example of a neuromuscular junction from a living mouse that was lightly labeled with r-α-bgt (<30% blocked) and then treated with a blocking dose of curare for 4 hours, at which point nearly 20% of its initial fluorescence intensity was lost. Over the next 4 hours, however, after curare had been washed out, the fluorescence intensity at the junction increased by 8%. Scale bar, 10 μm. (**B**) The same trend shown in (A) was seen in each of 15 junctions summarized in the graph. (**C**) A three-compartment model suggesting the way activity affects AChR accumulation and loss from neuromuscular junctions. In normal muscle fibers (*1*), the perijunctional region (P) serves as an intermediary way station between the internalized pool of receptors (I_{new} and I_{deg}) and the junctional pool (J). In the normal steady-state situation, the rate of receptor insertion into the perijunctional pool (k_1) must equal the rate of receptor internalization (k_2), and the rate of receptor migration from the perijunctional pool to the junction (k_3) must equal the rate of receptor migration from the junctional pool to the perijunctional region (k_4). In the absence of muscle activity (*2*), the trend favors k_4 and, within a few hours, an increase also in k_2. Resumption of activity (*3*) tilts the balance in the opposite direction, when k_1 and k_3 are increased. Question marks denote aspects of this model that have not yet been resolved by experiments.

lack of Ca^{2+} entry is the signal that initiates receptor loss (or prevents receptor stabilization) after receptor blockade because the AChR allows entry of calcium in addition to sodium and potassium. Ca^{2+} influx into the postsynaptic cell is also involved in the induction of both long-term potentiation and long-term depression (LTP and LTD) (*25*). One model of LTP ascribes the increase in efficacy to a recruitment of glutamate receptors to the synapse (*26*). Conversely, studies of LTD have suggested that rapid migration of glutamate receptors away from synapses explains the loss of synaptic strength (*27*). LTD and LTP may therefore be a neuronal equivalent of the reversible changes in receptor density after blockade and resumption of synaptic transmission at the neuromuscular junction.

The rapid loss of receptors from blocked synapses may serve to clear an intoxicated junction of nonfunctional receptors. That the loss of receptors is not accompanied by an equivalent increase in insertion of new receptors suggests that the muscle will not send large numbers of receptors to the junction when they will be immediately inactivated (perhaps permanently, if by snake toxin). Accelerated AChR loss might therefore have devastating effects on junctions that are blocked for long periods. In fact, patients on ventilators who are treated with reversible neuromuscular blocking agents for periods greater than 1 to 2 days sometimes develop a paralytic syndrome thought to be due to a defect in neuromuscular transmission that lasts days or even weeks after the blocking agents are removed (*28*). If this paralysis is caused by a decrease in receptor density at chronically blocked neuromuscular junctions, then muscle stimulation even in the presence of blocking agents may be useful in preventing such loss.

References and Notes

1. D. M. Fambrough and H. C. Hartzell, *Science* **176**, 189 (1972); H. C. Fertuck and M. M. Salpeter, *Proc. Natl. Acad. Sci. USA* **71**, 1376 (1974); *J. Cell Biol.* **69**, 144 (1976).
2. P. Ravdin and D. Axelrod, *Anal. Biochem.* **80**, 585 (1977).
3. S. G. Turney, S. M. Culican, J. W. Lichtman, *J. Neurosci. Methods* **64**, 199 (1996).
4. M. M. Rich and J. W. Lichtman, *J. Neurosci.* **9**, 1781 (1989); R. J. Balice-Gordon and J. W. Lichtman, *ibid.* **13**, 834 (1993); *Nature* **372**, 519 (1994).
5. Sternomastoid muscles were fixed in 4% paraformaldehyde and after extensive washing were labeled with r-α-bgt. In these junctions, loss of fluorescence intensity over hours or days was negligible (loss of 0.01 to 0.02% per hour, $t_{1/2}$ = ~5 months; n = 29). In addition, the results reported here show that the loss of fluorescence was related to muscle fiber activity, ruling out bleaching or unbinding as the explanation of the loss.
6. E. F. Stanley and D. B. Drachman, *Science* **222**, 67 (1983); D. A. Ramsay, D. B. Drachman, R. J. Drachman, E. F. Stanley, *Brain Res.* **581**, 198 (1992).
7. α-Bungarotoxin (15 to 20 μg/ml of Ringer's solution) or curare (2.5 mg/ml) or both were used to block neuromuscular transmission for the duration of the experiment by placing a coverslip over the exposed muscle to prevent drying and by replacing the fluid with additional blocking solution once per hour.
8. A mixture (50:50) of rhodamine-tagged and unlabeled α-bgt was used both to label the muscle and to maintain the neuromuscular blockade.
9. Based on the facts that (i) one bgt molecule inactivates an AChR, (ii) all receptors can bind two bgt molecules and (iii) binding of each α-bgt is independent, we calculated the relation between fluorescence label and percentage of receptors blocked. For example, application of r-α-bgt for 45 min labels 50% of the maximum fluorescence intensity, which means that 68% of receptors are blocked. In the experiments with a nonblocking dose of α-bgt, we blocked 20 to 40% of the receptors by labeling ~13 to 24% of the maximum fluorescence, as this degree of blockade is well below the 70% needed to impede action potentials in the muscle [C. J. Lingle and J. H. Steinbach, *Int. Anesthesiol. Clin.* **26**, 288 (1988)].
10. The ~14-day half-life is slightly longer than previous estimates of normal receptor turnover based on saturation with radiolabeled α-bgt (*7*). This difference is probably because the short period of increase AChR loss after receptor saturation (Fig. 1) was not previously noted.
11. In junctions saturated with labeled α-bgt such that all the receptors were associated with two bgt molecules, the loss of label was substantially slower on the second day after application than on the first day (Fig. 1). Moreover, when curare was applied to these muscles on the second day, the loss rate of the previously labeled receptors rapidly increased to ~5% per hour (n = 20). Because curare cannot bind to AChRs that already have two α-bgt molecules bound [D. X. Fu and S. M. Sine, *J. Biol. Chem.* **269**, 26152 (1994)], neuromuscular blockade rather than receptor occupation by blocking ligands induced accelerated receptor loss from the neuromuscular junction.
12. If one assumes both slowly degrading and rapidly degrading receptors at denervated junctions [S. L. Shyng and M. M. Salpeter, *J. Cell Biol.* **108**, 647 (1989)] and a half-life of 14 days (from the observations reported here) for slowly degraded AChRs, the result is a combined degradation half-life of 2.8 days at 1 week after denervation, which is very close to the half-life of 2.7 days found here.
13. J. S. Andreose, R. Xu, T. Lomo, M. M. Salpeter, G. Fumagalli [*J. Neurosci.* **13**, 3433 (1993)] previously showed that the acceleration in the rate of receptor loss after denervation was also blocked by electrical stimulation.
14. M. Akaaboune, S. M. Culican, S. G. Turney, J. W. Lichtman, data not shown.
15. Perijunctional receptors were imaged with 1.4 numerical aperture objectives and high-intensity laser illumination on a confocal scanning light microscope (Bio-Rad 1024). The gain was intentionally set high to allow the faint perijunctional signal to be seen (Fig. 3). These muscles were fixed in 4% paraformaldehyde, washed in saline overnight (to remove curare in the blocked preparations), and stained with r-α-bgt (5 μg/ml) for 30 min.
16. H. C. Hartzell and D. M. Fambrough, *J. Gen. Physiol.* **60**, 248 (1972); M. Stya and D. Axelrod, *J. Neurosci.* **4**, 70 (1984).
17. J. R. Sanes and J. W. Lichtman, *Annu. Rev. Neurosci.* **22**, 389 (1999).
18. M. M. Salpeter and R. Harris, *J. Cell Biol.* **96**, 1781 (1983); M. M. Salpeter, M. Marchaterre, R. Harris, *ibid.* **106**, 2087 (1988).
19. M. M. Salpeter and R. H. Loring, *Prog. Neurobiol.* **25**, 297 (1985).
20. S. M. Culican *et al.*, in preparation.
21. T. A. Levitt, R. H. Loring, M. M. Salpeter, *Science* **210**, 550 (1980); R. S. Brett, S. G. Younkin, M. Konieczkowski, R. M. Slugg, *Brain Res.* **233**, 133 (1982).
22. M. J. Dennis and R. Miledi, *J. Physiol. (London)* **237**, 431 (1974).
23. R. A. McKinney, M. Capogna, R. Durr, B. H. Gahwiler, S. M. Thompson, *Nature Neurosci.* **2**, 44 (1999).

24. S. Rotzler, H. Schramek, H. R. Brenner, *Nature* **349**, 337 (1991); J. L. McManaman, J. C. Blosser, S. H. Appel, *J. Neurosci.* **1**, 771 (1981).
25. D. J. Perkel, J. J. Petrozzino, R. A. Nicoll, J. A. Connor, *Neuron* **11**, 817 (1993); J. A. Cummings, R. M. Mulkey, R. A. Nicoll, R. C. Malenka, *ibid.* **16**, 825 (1996).
26. T. Manabe, P. Renner, R. A. Nicoll, *Nature* **355**, 50 (1992).
27. D. V. Lissin, R. C. Carroll, R. A. Nicoll, R. C. Malenkavon, M. Zastrow, *J. Neurosci.* **15**, 1263 (1999); R. C. Carroll, D. V. Lissin, M. V. Zastrow, R. A. Nicoll, R. C. Malenka, *Nature Neurosci.* **2**, 454 (1999).
28. J. L. Gooch, M. R. Suchyta, J. M. Balbierz, J. H. Petajan, T. P. Clemmer, *Crit. Care Med.* **19**, 1125 (1991); V. Segredo *et al.*, *N. Engl. J. Med.* **327**, 524 (1992); T. L. Kurt, *J. Toxicol. Clin. Toxicol.* **34**, 253 (1996).
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REPORTS

Role of Metal-Oxide Interface in Determining the Spin Polarization of Magnetic Tunnel Junctions

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The role of the metal-oxide interface in determining the spin polarization of electrons tunneling from or into ferromagnetic transition metals in magnetic tunnel junctions is reported. The spin polarization of cobalt in tunnel junctions with an alumina barrier is positive, but it is negative when the barrier is strontium titanate or cerium lanthanite. The results are ascribed to bonding effects at the transition metal–barrier interface. The influence of the electronic structure of metal-oxide interfaces on the spin polarization raises interesting fundamental problems and opens new ways to optimize the magnetoresistance of tunnel junctions.

A tunnel junction consists of two metallic layers (electrodes) separated by a thin insulating layer. When the electrodes are ferromagnetic, the tunneling of electrons across the insulating barrier is spin-polarized, and this polarization reflects that of the density of states (DOS) at the Fermi level (E_F) of the electrodes. This spin polarization is the origin of the tunneling magnetoresistance (TMR), which is currently a hot topic of research in magnetism (*1*) and very promising for applications (*2*). Paradoxically, even though applications have already begun to be developed, there are still gaps in our understanding of spin-polarized tunneling. For example, the physics governing the spin polarization of tunneling electrons is not clearly understood. Previously, the spin polarization P of electrons tunneling from a given ferromagnetic electrode was generally thought to reflect a characteristic intrinsic spin polarization of the DOS in the ferromagnet,

$$P = \frac{N_\uparrow(E_F) - N_\downarrow(E_F)}{N_\uparrow(E_F) + N_\downarrow(E_F)} \quad (1)$$

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However, recent findings show that the amplitude of the spin polarization, and even its sign, depends on the choice of barrier material (*3, 4*). Here, we describe a series of TMR experiments on Co/I/$La_{0.7}Sr_{0.3}MnO_3$ (LSMO) tunnel junctions, where the barrier I can be $SrTiO_3$ (STO), $Ce_{0.69}La_{0.31}O_{1.845}$ (CLO), or Al_2O_3 (ALO). The effective polarization of Co was found to be positive (higher tunneling probability for majority spin electrons) when I is ALO, and negative (higher tunneling probability for minority spin electrons) when I is STO or CLO. Moreover, the bias dependence of the TMR is completely different in these two cases. The strong influence of the electronic structure of the barrier and barrier-electrode interface in tunnel junctions raises interesting fundamental problems and presents new ways to tailor the TMR.

The first piece of information on the spin polarization of electrons tunneling from a ferromagnetic metal (F) comes from experiments on F/I/S junctions, in which the second electrode is a superconductor (S). The spin splitting of the quasi-particle DOS of S, induced by a magnetic field, can be used to analyze the spin polarization of the tunneling current. Extensive data have been obtained with F/ALO/Al junctions, and a positive polarization has been found for all the ferromagnetic metals and alloys that have been studied (*5*). This is surprising, especially for metals like Co or Ni in which a negative polarization is expected from the smaller DOS at E_F for the majority spin direction (the majority spin d subband is below E_F). This problem has not been clearly solved, even though it is frequently argued that s-character electrons should tunnel more easily, so that the experimental positive polarization can reflect only that of the s-character DOS (*6, 7*). Some theoretical justification has been provided by ab initio calculations of the electronic structure at a Co-ALO interface. Nguyen-Mahn *et al.* (*8*) determined the DOS of the tunneling electrons on the first Al atoms at the interface and found that, because of an sp-d bonding mechanism between Al and Co, this DOS is positively polarized. This can be viewed as an interface filtering effect controlling the starting point of the polarized evanescent wave in the barrier.

In junctions with two ferromagnetic electrodes, $F_1/I/F_2$, spin-polarized tunneling gives rise to TMR because the resistance of the junction depends on whether the electrodes have parallel or antiparallel magnetizations. This change can be large, typically 15 to 40% at room temperature, so that TMR has great relevance for the technology of MRAM (magnetic random access memory) or read heads. The experimental results at low bias are generally interpreted according to Jullière's expression,

$$\frac{\Delta R}{R} = \frac{R_{AP} - R_P}{R_{AP}} = \frac{2P_1P_2}{1 + P_1P_2} \quad (2)$$

where R_{AP} and R_P are the resistances in the antiparallel and parallel states, respectively, and P_1 and P_2 are the electron spin polarizations of the two electrodes.

In junctions studied up to now, mostly with ferromagnetic transition-metal electrodes and ALO barriers, a normal TMR has been found; that is, the tunnel resistance is smaller when the magnetizations of F_1 and F_2 are parallel. This behavior is expected when the sign of the polarization coefficient P is the same for both electrodes and is consistent with the aforementioned uniformly positive spin polarization found for various transition metals in F/ALO/Al junctions. However, two recent results have indicated that, with types of barrier other than ALO, the spin polarization of electrons tunneling from Co or NiFe (permalloy) can also be negative. Sharma *et*

References and Notes

[1] **Acetylcholine Receptors: Number and Distribution at Neuromuscular Junctions in Rat Diaphragm**

Douglas M. Fambrough; H. Criss Hartzell

Science, New Series, Vol. 176, No. 4031. (Apr. 14, 1972), pp. 189-191.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819720414%293%3A176%3A4031%3C189%3AARNADA%3E2.0.CO%3B2-I

[1] **Localization of Acetylcholine Receptor by ¹²⁵ I-Labeled α-Bungarotoxin Binding at Mouse Motor Endplates**

Helen C. Fertuck; Miriam M. Salpeter

Proceedings of the National Academy of Sciences of the United States of America, Vol. 71, No. 4. (Apr., 1974), pp. 1376-1378.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%28197404%2971%3A4%3C1376%3ALOARBB%3E2.0.CO%3B2-Q

[6] **Rapid Degradation of ``New'' Acetylcholine Receptors at Neuromuscular Junctions**

Elis F. Stanley; Daniel B. Drachman

Science, New Series, Vol. 222, No. 4619. (Oct. 7, 1983), pp. 67-69.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819831007%293%3A222%3A4619%3C67%3ARDO%60AR%3E2.0.CO%3B2-5

LINKED CITATIONS

[21] **Neuronal Control of Acetylcholine Receptor turnover Rate at a Vertebrate Neuromuscular Junction**

Tami A. Levitt; Ralph H. Loring; Miriam M. Salpeter

Science, New Series, Vol. 210, No. 4469. (Oct. 31, 1980), pp. 550-551.

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