

Terminal Sprouting Is Not Responsible for Enhanced Transmitter Release at Disused Neuromuscular Junctions of the Rat

Tetsuhiro Tsujimoto, Masashi Umemiya, and Motoy Kuno

Department of Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan

Chronic block of nerve–muscle activity is known to induce sprouting of motor nerve terminals and to enhance transmitter release at the neuromuscular junction. Increased transmitter release has been assumed to be a physiological correlate of disuse-induced sprouting of nerve terminals. We examined this assumption in the rat extensor digitorum longus muscle following chronic conduction block of the sciatic nerve with TTX. The minimal period of nerve block required for the expression of terminal sprouting was 3 d, whereas transmitter release, measured by the quantal analysis of end-plate potentials, was already enhanced within 24 hr of nerve block. Following 6 d of nerve block, sprouting was observed in about 35% of the motor nerve terminals examined. Under this condition, the total length of individual terminals was significantly greater in the terminals with sprouts than those without sprouts. However, enhancement of transmitter release occurred uniformly at these junctions regardless of the presence or absence of terminal sprouts. Also, transmitter release enhanced by nerve block for 2 d remained elevated for at least 4 d even after resumption of nerve activity without the formation of terminal sprouts. It is concluded that terminal sprouting and increased transmitter release induced in disused neuromuscular junctions are not causally related and that the signals for inducing these 2 events are at least quantitatively different.

Prolonged elimination of presynaptic impulse activity causes enhancement of synaptic transmission in spinal motoneurons (Gallego et al., 1979; Manabe et al., 1989), sympathetic ganglia (Gallego and Geijo, 1987), and neuromuscular junctions (Snider and Harris, 1979; Harris, 1981; Tsujimoto and Kuno, 1988). At neuromuscular junctions, the same procedure also induces sprouting of motor nerve terminals (Brown and Ironton, 1977; Pestronk and Drachman, 1978; Betz et al., 1980; Harris, 1981; Tsujimoto and Kuno, 1988; Diaz and Pecot-Dechavassine, 1989). Because of apparent concurrence of the 2 events, an increase in transmitter release observed at neuromuscular junctions following prolonged block of nerve–muscle activity has been considered to be a physiological correlate of terminal sprouting (Snider and Harris, 1979; Harris, 1981). Sprouting of motor nerve terminals is also observed in the muscle whose activity is partially reduced following transection of the spinal cord (Eldridge et al., 1981) or in some neurological disease

(Brownell et al., 1972; Coërs and Telerman-Toppet, 1976). Disuse-induced sprouting may then represent an adaptive response which could help to restore functional transmission and muscle activity (Grinnell and Herrera, 1981).

The amount of transmitter released at a neuromuscular junction is normally proportional to the total length of its nerve terminal (Kuno et al., 1971; Grinnell and Herrera, 1980, 1981) or to the total active zone length (Propst and Ko, 1987). Therefore, it is possible that an increase in the terminal length by the formation of sprouts at disused junctions may result in enhancement of transmitter release. In fact, sprouts arising from motor nerve terminals have been shown to contain numerous synaptic vesicles (Duchen, 1971). Also, long-term sensitization observed in *Aplysia* (Bailey and Chen, 1988a, b, 1989) or long-term facilitation induced at the crayfish neuromuscular junction (Lnenicka et al., 1986) appears to be associated with increased number of synaptic contacts or varicosities. Thus, the expression of terminal sprouting may provide the morphological basis for functional plasticity at neuromuscular junctions.

In a previous study (Tsujimoto and Kuno, 1988), we found that the formation of sprouts of motor nerve terminals following chronic block of nerve–muscle activity can be suppressed by daily treatment with calcitonin gene-related peptide (CGRP). However, CGRP treatment did not prevent increased transmitter release at the disused neuromuscular junction (Tsujimoto and Kuno, 1988). Therefore, terminal sprouting and enhancement of transmitter release can be dissociated at least under certain conditions. A question may then arise as to whether terminal sprouting is indeed responsible for increased transmitter release at disused junctions. We addressed this question in the present study. The results show that these 2 events are not causally related.

Materials and Methods

Preparation. Wistar rats, 180–250 gm body weight, were used. Both the electrophysiological and morphological observations were made on the extensor digitorum longus (EDL) muscle in the hind leg following chronic conduction block of the sciatic nerve. The conduction block was achieved by implantation of a glass capillary filled with a TTX solution beneath the epineurium of the right sciatic nerve in a manner described previously (Tsujimoto and Kuno, 1988). On the contralateral side, a similar capillary filled with the vehicle solution alone (citrate buffer without TTX) was applied to the sciatic nerve, and this unblocked side served as a control. These operations were performed under pentobarbital anesthesia. The conduction block of the sciatic nerve on the experimental side was maintained for 0.5–10 d. Nerve block was assessed twice daily by examining the toe-spreading reflex, which was absent on the side treated with TTX (Tsujimoto and Kuno, 1988).

Electrophysiology. The EDL muscles excised from the control and experimental sides under pentobarbital anesthesia were placed side by side in the same recording chamber. The preparations were superfused

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Correspondence should be addressed to Motoy Kuno at the above address.

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with an oxygenated (95% O₂–5% CO₂) solution with the following composition (in mM): NaCl, 136.8; KCl, 5.0; CaCl₂, 1.0; MgCl₂, 8.0; NaH₂PO₄, 1.0; NaHCO₃, 12.0. The amount of transmitter release was evaluated by the quantal analysis of end-plate potentials (epps) as described previously (Tsujimoto and Kuno, 1988). Briefly, the 2 muscle nerves were stimulated separately with suction electrodes at a frequency of 0.5 Hz. Intracellular recordings were made alternately from the 2 muscles with glass microelectrodes filled with 3 M KCl, sampling about 10 junctions from the corresponding area of each muscle. At each junction, a large number of epps and spontaneous miniature epps (mepps) were recorded, and the mean quantum content was calculated from the ratio of the mean epp amplitude to the mean mepp amplitude. The bath temperature was maintained at 25.0 ± 1.0°C.

In a series of experiments, the size of motor nerve terminal was measured in the same junctions as those subjected to the quantal analysis. For this purpose, the muscle fiber recorded was marked with 5(6)-carboxyfluorescein (Kodak; Purves et al., 1986). The intracellular electrode was filled with a 5% solution of carboxyfluorescein dissolved in 2 M potassium citrate (pH adjusted to 8.0 by KOH and HEPES). After electrophysiological studies, the fluorescent dye was injected into the muscle fiber by applying hyperpolarizing currents for 10 min. The iontophoretic current was a 100 Hz sine wave which was half-wave rectified to exclude the depolarizing phase (peak intensity, 30 nA). Immediately after the experiment, the muscle was fixed with 2.5% glutaraldehyde for 10 sec, and the marked muscle fibers were isolated under the dissecting microscope. Carboxyfluorescein-containing fibers and their end-plates could be seen clearly when the muscle was illuminated by blue light with a wavelength between 420 and 490 nm and viewed through a green interference filter. The brief fixation with glutaraldehyde was weak enough to prevent the dye leakage from the muscle fiber but strong enough to facilitate fiber dissection. The specimens were then stained for the motor nerve terminals 24 hr later as described below.

Morphology. The muscle used for electrophysiological studies was fixed for 40 sec (30 sec in the case of dissected muscle fibers, see above) with 2.5% glutaraldehyde in 0.1 M PIPES buffer. The motor nerve terminals were stained with nitro blue tetrazolium and phenazine methylsulfate (Letinsky and DeCino, 1980). Single muscle fibers in the superficial layer were dissected and mounted in glycerol on glass slides. The entire terminal profile was reconstructed from several photomicrographs taken at different focal planes for each junction. Morphometric measurements were made by a digitizing tablet attached to a computer. The original end-plate region was determined by the demarcation of Schwann cell processes identified with Nomarski optics, and those terminal processes that extended beyond the end-plate region were considered as ultraterminal sprouts. The end-plate region determined by this method agreed with that identified by staining for AChE, and further technical details have also been given previously (Tsujimoto and Kuno, 1988).

All values presented in the text give the mean ± SD. The statistical analysis was made by *t*-tests with the significance limit of *p* < 0.05.

Results

Time course of sprouting and enhanced transmitter release induced by nerve block

Figure 1*A* shows changes in the ratio of the number of terminals with sprouts to the total number of junctions examined in each EDL muscle as a function of the duration of conduction block of the sciatic nerve with TTX. In each muscle, about 30 (25–41) junctions were sampled for morphometric studies of motor nerve terminals. The mean control value shown at day 0 in Figure 1*A* was obtained from motor nerve terminals examined in 18 EDL muscles on the control, nerve-unblocked side (see Materials and Methods). As reported previously (Barker and Ip, 1966; Tuffery, 1971; Brown and Ironton, 1977; Betz et al., 1980; Harris, 1981; Lichtman et al., 1987; Tsujimoto and Kuno, 1988), a small population of nerve terminals showed sprouting even in normally active muscles. In the 18 control muscles, terminal sprouts were observed in 3.3 ± 2.0% of the junctions examined (range, 0–7.1%; indicated by shaded area in inset of Fig. 1*A*). When the nerve conduction had been blocked for 3 d, 2 of the 3 EDL muscles examined on the experimental side showed higher

ratios of terminals with sprouts than the normal range, whereas this ratio was within the normal range in all the 3 muscles observed after 2 d of nerve block (Fig. 1*A*, inset). As shown in Figure 1*A*, the ratio of terminals with sprouts increased further as the duration of nerve block had been prolonged, reaching 86% after 10 d of nerve block. Thus, the minimal period of nerve block necessary for the expression of terminal sprouting appears to be about 3 d. This was in good agreement with the value (3.5 d) reported for the mouse hind leg muscle (Brown and Ironton, 1977).

Figure 1*B* shows the time course of an increase in transmitter release measured in terms of the frequency of mepps following conduction block of the sciatic nerve. In each animal, the ratio of the mean of the mepp frequencies observed in about 10 junctions on the experimental (nerve-blocked) side to that on the control side was obtained. Each point in Figure 1*B* shows the average ratio from 3 rats. Following conduction block for 12 hr, there was no significant difference in transmitter release between the control and experimental sides. A significant increase in transmitter release on the experimental side was already seen after 24 hr of conduction block (Fig. 1*B*). Enhancement of transmitter release was maintained or further augmented when the duration of conduction block had been prolonged. As noted by Bray et al. (1979), the resting potential of muscle fibers was significantly lower on the nerve-blocked side than on the control side when the nerve conduction had been blocked for more than 4 d. After 4 d of nerve block, for example, the mean resting potential on the nerve-blocked side was -65 ± 10 mV, whereas that on the control side was -72 ± 9 mV (also, see below).

Chronic block of nerve–muscle activity produces general enlargement of the motor nerve terminals, in addition to the formation of sprouts (Brown and Ironton, 1977; Pestronk and Drachman, 1978; Betz et al., 1980; Harris, 1981; Tsujimoto and Kuno, 1988). Therefore, the total length of individual terminals, including sprouts if present, was measured. The mean total terminal length on the control side ranged from 113 to 173 μm (average, 150 ± 23 μm) in different animals. On the experimental side, this range was from 124 to 321 μm (average, 178 ± 48 μm). Figure 2*A* shows changes of the mean total terminal length on the experimental side relative to that on the control side as a function of the duration of nerve block. The relative increment in the total terminal length on the experimental side was significant when the conduction block had been maintained for 4 d or more (Fig. 2*A*). Figure 2*B* shows the time course of increases in transmitter release measured in terms of the mean quantum content. The results were similar to those measured by the frequency of mepps (Fig. 1*B*). Again, transmitter release was significantly increased when nerve conduction had been blocked for more than 24 hr (Fig. 2*B*). As reviewed by Rotshenker (1988), sprouting of motor nerve terminals may be induced in normally innervated muscle by denervation of the corresponding muscle on the contralateral side. In the present study, there was no systematic change in the proportion of terminals with sprouts (0–7%) or in the mean quantum content (0.8–2.4) at the control junctions contralateral to the nerve-blocked side as a function of the duration of conduction block.

From these results it seems clear that, when nerve–muscle activity is chronically eliminated, increased transmitter release is expressed before the formation of terminal sprouts or enlargement of terminal size at neuromuscular junctions. Also, chronic conduction block of the sciatic nerve did not cause any

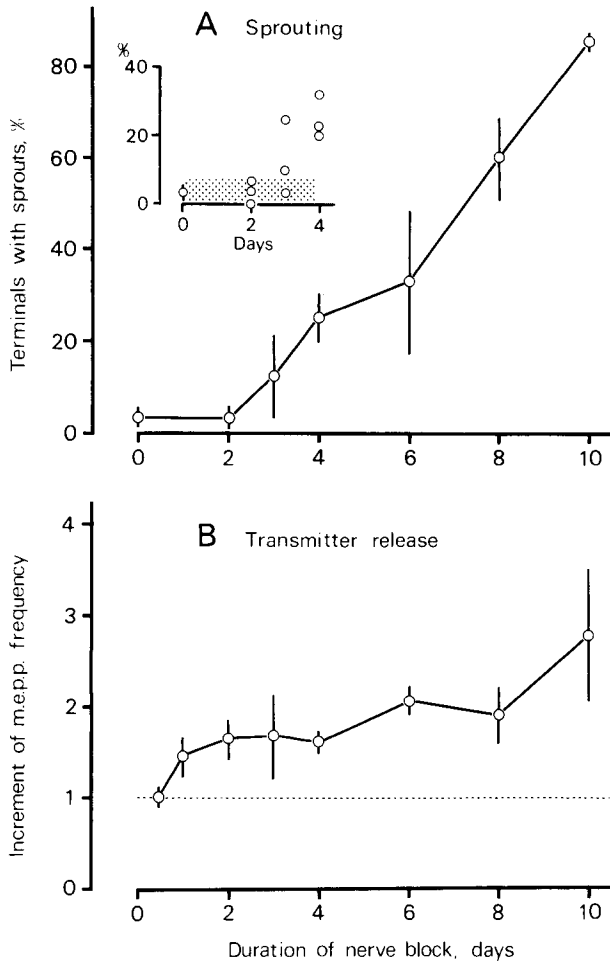


Figure 1. Time course of the formation of sprouts and enhancement of transmitter release induced by chronic block of nerve-muscle activity. Abscissae, days of conduction block of the sciatic nerve with TTX. *A*, Changes in the ratio of the number of terminals with sprouts to the total number of junctions examined. Each point represents the mean (\pm SD) from 3 rats, except for the control value at day 0, which is the mean from 18 normally active muscles. *Inset*, Mean values from individual muscles examined after 2–4 d of nerve block are plotted separately. *Shaded area* indicates the range of the ratio of terminals with sprouts observed in normally active muscles. *B*, Relative changes in the frequency of mepps observed on the nerve-blocked side to that on the control side. Each point, mean from 3 rats.

increase in the thickness (diameter) of motor nerve terminals (e.g., see figure 1 in Tsujimoto and Kuno, 1988). Therefore, morphological alterations induced under these conditions are not a prerequisite to enhancement of transmitter release.

Transmitter release from nerve terminals with or without sprouts

Enhancement of transmitter release after nerve block can occur without terminal sprouting. However, it remains uncertain whether the formation of sprouts may contribute to an increase in transmitter release. As shown in Figure 1*A*, when nerve conduction had been blocked for 6 d, terminal sprouting was observed in approximately 35% of the junctions examined. One might then ask whether transmitter release may be greater at the junctions with terminal sprouts than those without sprouts under this condition. To address this question, electrophysiological studies were followed by morphometric observations on

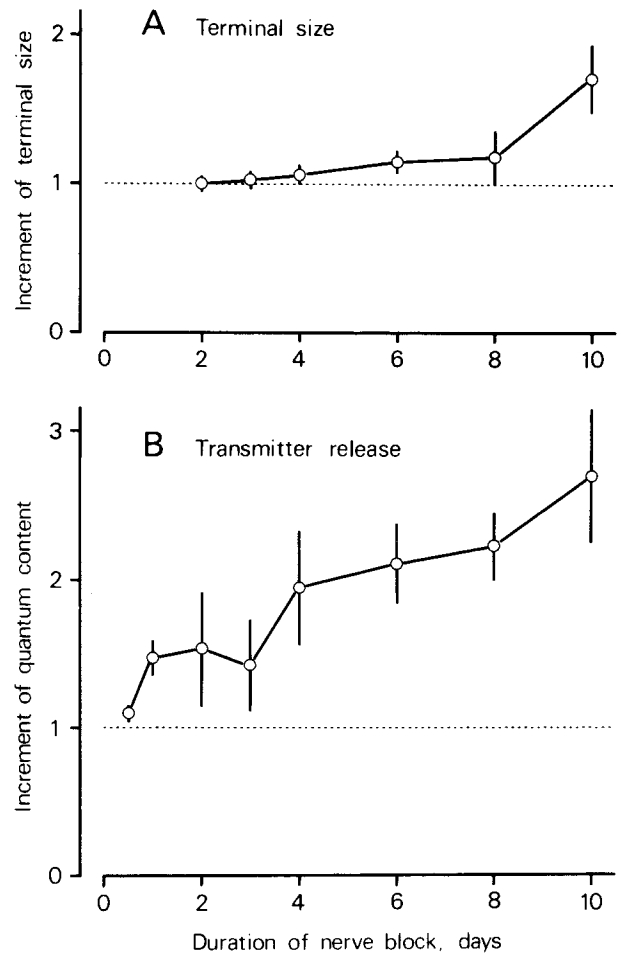


Figure 2. Changes in the total terminal length and transmitter release as a function of the duration of nerve block. *A*, Relative changes in the mean total terminal length observed on the nerve blocked side to that on the control side. Each point represents the mean from 3 rats. *B*, Same as in Figure 1*B*, but transmitter release measured in terms of the mean quantum content of epps.

the motor nerve terminals at the same junctions identified with carboxyfluorescein (see Materials and Methods).

Figure 3*A* shows the relation between the total terminal length and the mean quantum content observed in individual neuromuscular junctions on the control side sampled from 14 different animals. In agreement with the observations on the amphibian neuromuscular junction (Kuno et al., 1971; Grinnell and Herrera, 1980, 1981; Propst and Ko, 1987), there was a significant ($p < 0.005$), positive correlation ($R = 0.64$) between the terminal length and the mean quantum content. A similar correlation ($p < 0.005$; $R = 0.44$) was also found between the terminal length and the frequency of mepps (Fig. 3*B*). Among the 36 junctions examined on the control side, one motor nerve terminal showed a sprout (Fig. 3, filled circles). This particular motor nerve terminal was the largest in the control junctions examined, although the sprout length (33 μ m) was only 14% of its total terminal length.

Figure 4 shows 2 examples of motor nerve terminals observed on the experimental side following 6 d of nerve block; one terminal (Fig. 4*A*) showed 2 sprouts, whereas the other (Fig. 4*B*) had no sprouts. In these particular examples, the muscle fiber that had a terminal with sprouts was larger in diameter than

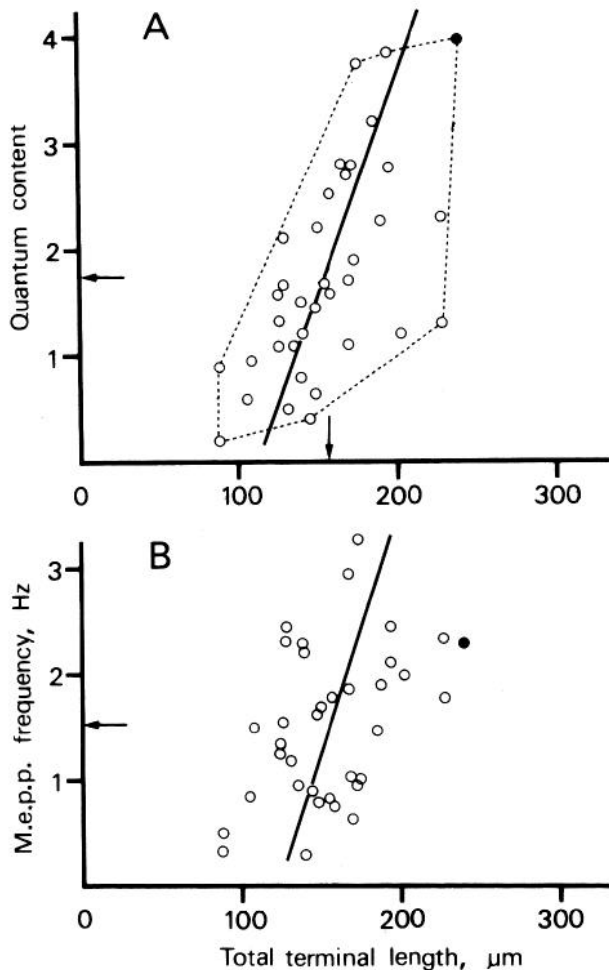


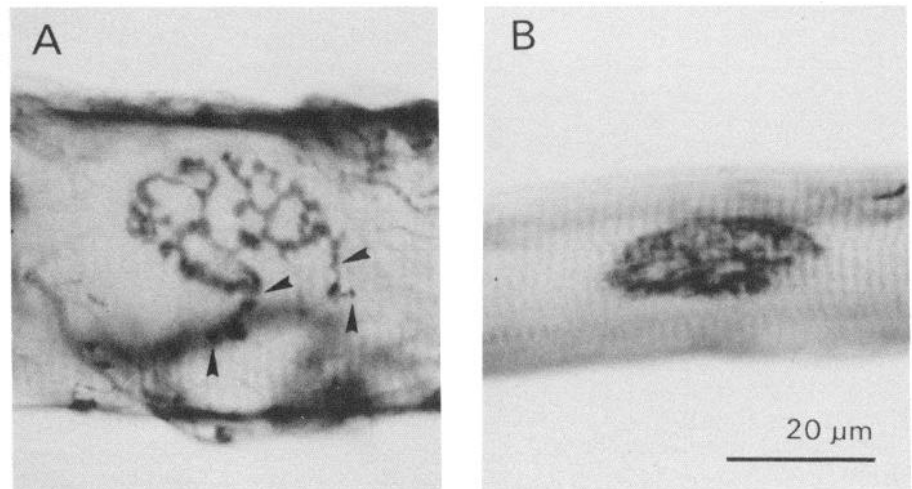
Figure 3. Relation between the total terminal length and the amount of transmitter release at individual neuromuscular junctions on the control side. *A*, Transmitter release measured in terms of the mean quantum content of epps. *B*, Transmitter release measured by the frequency of mepps. Filled circles, a terminal with sprouts; solid lines, linear-regression lines calculated by the method of least-squares; arrows, mean values.

the one that had a terminal without sprouts. On the average, however, there was no significant difference in diameter between muscle fibers that had motor nerve terminals with sprouts ($44 \pm 11 \mu\text{m}$; $n = 32$) and those without sprouts ($44 \pm 11 \mu\text{m}$; n

$= 25$). These mean diameters of muscle fibers on the nerve-blocked side were significantly ($p < 0.005$) smaller than the mean muscle fiber diameter ($53 \pm 8 \mu\text{m}$; $n = 36$) observed on the control side. The mean resting membrane potential of muscle fibers on the nerve-blocked side was significantly lower than that on the control side ($-72 \pm 7 \text{ mV}$). However, again there was no significant difference in resting potential between the muscle fibers which had terminals with sprouts ($-61 \pm 8 \text{ mV}$) and those without sprouts ($-60 \pm 6 \text{ mV}$). Thus, there was no apparent tendency for muscle fibers having terminals with sprouts to undergo more atrophic changes than those without sprouts.

The mean quantum content measured in the junction illustrated in Figure 4*A* was 3.2 and that shown in Figure 4*B* was 4.2. Figure 5 summarizes the relationship between terminal length and mean quantum content observed on the experimental side in which the nerve conduction had been blocked for 6 d. The results obtained from the junctions with terminal sprouts (Fig. 5*A*) and those from the junctions without sprouts (Fig. 5*B*) are plotted separately. For comparison, the range observed on the control side (Fig. 3*A*) is depicted by dotted-line boundaries. The mean terminal length in the junctions with sprouts ($196 \pm 35 \mu\text{m}$, $n = 32$; arrow in Fig. 5*A*) was significantly ($p < 0.005$) larger than the control value ($156 \pm 36 \mu\text{m}$, $n = 36$; arrow in Fig. 3*A* and arrowheads in Fig. 5). In contrast, the mean terminal length in the junctions without sprouts ($166 \pm 36 \mu\text{m}$, $n = 25$; arrow in Fig. 5*B*) was not significantly ($0.30 > p > 0.20$) different from the control value. Despite the difference in total length between the terminals with and without sprouts, the average mean quantum content in the junctions with sprouts (2.9 ± 1.3 ; arrow in Fig. 5*A*) was the same as that in the junctions without sprouts (2.9 ± 1.2 ; arrow in Fig. 5*B*). These values were significantly ($p < 0.005$) higher than the mean quantum content observed on the control side (1.8 ± 1.0 ; arrow in Fig. 3*A* and arrowheads in Fig. 5). Thus, the amount of transmitter release was significantly enhanced on the experimental side, regardless of the presence or absence of terminal sprouts. Also, increased transmitter release was observed at disused junctions without a significant increase in terminal size (Fig. 5*B*). This again indicates that an increase in transmitter release and terminal enlargement induced at disused junctions are not causally related. Furthermore, it should be noted that the positive correlation between the terminal length and the mean quantum content observed in the control junctions (Fig. 3*A*) is no longer present at disused junctions irrespective of whether or not the terminals

Figure 4. Photomicrographs of motor nerve terminals observed after 6 d of nerve block. *A*, Terminal with sprouts. The origin and tip of sprouts are shown by oblique and upward-pointing arrowheads, respectively. *B*, Terminal without sprouting.



had sprouts [$R = 0.06$ (Fig. 5A) $R = 0.12$ (Fig. 5B), respectively]. This also implies that prolonged disuse causes an increase in transmitter release at neuromuscular junctions by a process that is independent of regulation of terminal size.

Dissociation of the expression for sprouting and enhancement of transmitter release

As shown in Figure 1, enhancement of transmitter release was expressed before the formation of terminal sprouts when nerve-muscle activity had been blocked. However, it is not clear whether this is due to a difference in latency of the expression between the 2 events in response to the same stimulus or whether the 2 events may be induced by quantitatively or qualitatively different stimuli. For example, it is possible that a nerve block for 1 or 2 d may be sufficient to induce terminal sprouting if a latency of more than 3 d is given to the junctions without a further nerve block. To test this, we examined transmitter release and the formation of sprouts at the neuromuscular junctions 4 d after removal of the TTX capillary that had been applied to the sciatic nerve for 2 d. By 12 hr after removal of the TTX capillary, the toe-spreading reflex on the experimental side had already recovered, indicating resumption of nerve activity. As shown in Figure 6A, transmitter release on the experimental side was still significantly ($p < 0.05$) enhanced compared with that on the control side. In contrast, there was no significant difference in the total terminal length between the control and experimental sides (Fig. 6B). About 30 junctions were sampled for the morphometric measurements in each EDL muscle on the control and experimental sides of 3 rats. No terminal sprouting was found on the control side ($n = 86$). On the experimental side, sprouting was observed at a single junction in each of 2 out of the 3 rats. This ratio of terminals with sprouts (2.3%, $n = 88$) was within the normal range (Fig. 1A, inset). Thus, the stimulus adequate to induce and maintain enhancement of transmitter release is not sufficient for inducing terminal sprouts even with appropriate latencies.

Discussion

The present study shows that an increase in transmitter release and terminal sprouting induced at neuromuscular junctions by chronic block of nerve-muscle activity are not causally related. First, increased transmitter release occurred before the expression of terminal sprouting. Second, following 6 d of nerve block, the junctions that had terminal sprouts and those that had no sprouts showed the same degree of enhancement of transmitter release. In normally active muscles of the rat, the amount of transmitter release at each junction was proportional to the total length of its motor nerve terminal as previously reported for the amphibian neuromuscular junction (Kuno et al., 1971; Grinnell and Herrera, 1980, 1981; Propst and Ko, 1987). After chronic block of nerve-muscle activity, however, this correlation was lost. This also indicates that enhancement of transmitter release at disused junctions is independent of alterations in the total length of motor nerve terminals.

There is little doubt that both terminal sprouting and enhancement of transmitter release are induced by chronic block of nerve-muscle activity. However, the expression of terminal sprouting required about 3 d of nerve block, instead of a 24 hr block for the acquisition of enhancement of transmitter release. Increased transmitter release observed following a 2 d nerve block was maintained for at least 4 d after resumption of the nerve-muscle activity. However, there was no sign of the for-

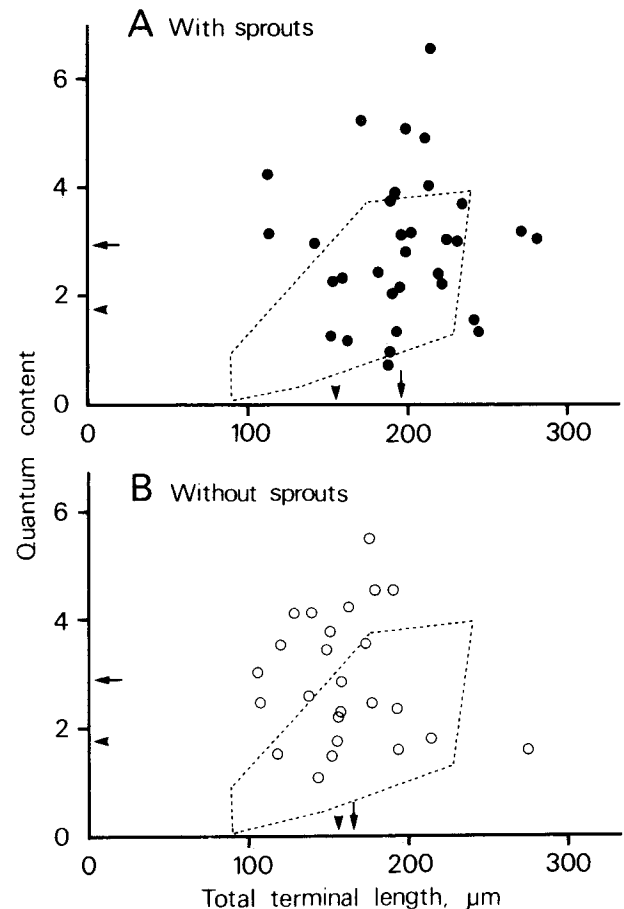


Figure 5. Relation between total terminal length and mean quantum content at individual junctions observed after 6 d of nerve block. *A*, Terminals with sprouts. *B*, Terminals without sprouts. Arrows, mean values; arrowheads, the mean values observed on the control side as indicated by arrows in Figure 3; Dotted-line boundaries, range of distribution observed on the control side as shown in Figure 3A.

mation of terminal sprouts under this condition. Therefore, the difference in the period of nerve block required for inducing the 2 events cannot be attributed only to the difference in latency of their expression. It could be argued that terminal sprouts once formed by nerve block may withdraw quickly following resumption of impulse activity. However, Harris (1981) has reported that an increase in the total terminal length induced by chronic nerve block is maintained even 4 weeks after resumption of nerve-muscle activity. Thus, the above result implies that the stimulus or signal for enhancement of transmitter release is different at least quantitatively, if not qualitatively, from that for terminal sprouting. The possible difference in the nature of the signals for the 2 events may account for the discriminative effect of CGRP, which blocks the formation of terminal sprouts without affecting increased transmitter release at disused junctions (Tsujimoto and Kuno, 1988). There are other examples in which functional plasticity occurs at neuromuscular junctions without the formation of terminal sprouts. Parnas et al. (1984), for example, have reported that elimination of one of the motor nerves to a lobster muscle increases the amount of transmitter release in response to stimulation of the remaining nerve before sprouting takes place. Also, increased transmitter release observed by stimulation of the intact motor fibers following partial denervation of the frog muscle occurs before any sign of sprouting (Grinnell, 1988).

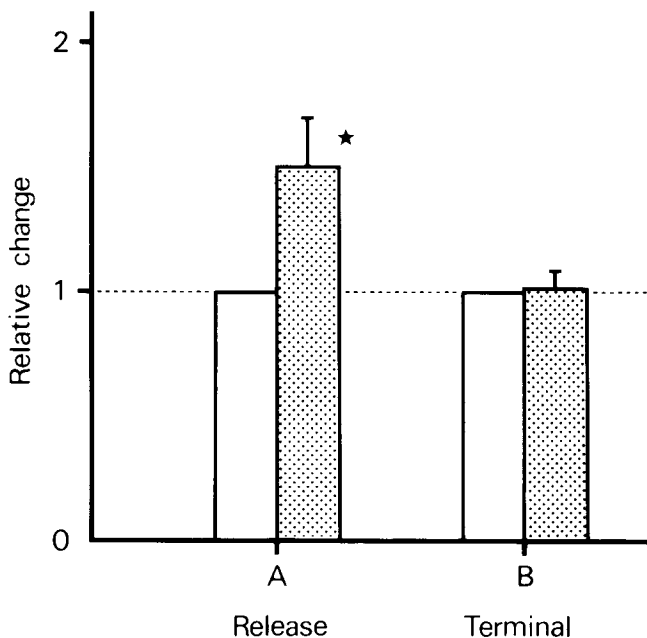


Figure 6. Changes in the mean quantum content (A) and the mean total terminal length (B) on the nerve-blocked side relative to those on the control side. The measurements were made 4 d after removal of the TTX capillary which had been applied to the sciatic nerve on the experimental side for 2 d. Vertical bars, SD measured in 3 rats; asterisk, significant difference between the control and experimental sides.

While the stimulus for terminal sprouting appears to arise primarily from inactivity of the muscle (Brown and Holland, 1979; Brown et al., 1980; Betz et al., 1980; Slack and Pockett, 1981; Pockett and Slack, 1982), it is not certain whether enhancement of transmitter release is associated with inactivity of the muscle or with inactivity of the nerve terminal. Since increased transmitter release is maintained for at least 4 d after resumption of nerve-muscle activity, this is apparently a long-term plasticity. It has been suggested that long-term synaptic plasticity requires the synthesis of new macromolecules (Montarolo et al., 1986, 1988). If this were the case, the block of nerve-muscle activity would be signaled to the motoneuron cell bodies, which, in turn, may dictate enhancement of transmitter release at their nerve terminals. It remains to be solved how such a bidirectional signal can be transferred within 24 hr of nerve block. Enhancement of synaptic transmission observed following elimination of presynaptic impulse activity also occurs within 1 or 2 d in sympathetic ganglia (Gallego and Geijo, 1987) and spinal motoneurons (Manabe et al., 1989). Gallego and Geijo (1987) have suggested that synaptic enhancement of sympathetic ganglia induced under this condition is associated with some alterations in the cell bodies of preganglionic neurons (also, see Miyata and Yasuda, 1988, for synapses on motoneurons).

How transmitter release is enhanced at disused neuromuscular junctions is not known. At regenerating neuromuscular junctions after nerve crush, a progressive increase in quantum content of epps precedes an increase in the frequency of mepps (Ding, 1982). At disused junctions, an increase in the quantum content occurred in parallel with that in the frequency of mepps (data not shown). Also, enhancement of transmitter release at disused junctions is not associated with any changes in the calcium-dependence of transmitter release or in the process of depolarization-release coupling (Harris, 1981). It is then pos-

sible that increased transmitter release at disused junctions may result from an increase in the number or length of active zones (Herrera et al., 1985; Propst and Ko, 1987). Since increased transmitter release is not associated with changes in the total terminal length or terminal diameter, the possible alteration may be an increase in the density of active zones. At a single neuromuscular junction, the probability of quantal release of transmitter is known to be substantially lower at release sites near the end of a terminal branch than at those sites near the point of nerve entry (D'Alonzo and Grinnell, 1985; Bennett et al., 1986). Disuse-induced enhancement of transmitter release may be due to a selective increase in release probability at these synaptic sites located near the ends of terminal branches. Yee and Pestronk (1987) have shown that ACh receptors are distributed along the outgrowth of motor nerve terminals within 7 days after muscle paralysis. However, it is not certain whether terminal sprouts may function as release sites of the transmitter.

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