Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 515–519, January 1978
Neurobiology

Genetic dissection of short-term and long-term facilitation at the *Drosophila* neuromuscular junction

(synaptic efficacy/neurological mutant)

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Communicated by Seymour Benzer, October 17, 1977

ABSTRACT **Transmitter release at the *Drosophila* larval neuromuscular junction may be increased by previous activity of the nerve. This facilitation phenomenon involves at least two processes, one short-term and the other long-term. These are shown to be based on different mechanisms because (*i*) a mutant was found that had abnormal long-term facilitation but normal short-term facilitation; and (*ii*) long-term facilitation was eliminated by tetrodotoxin or by removing external Na^+ but short-term facilitation was not. In long-term facilitation, there was a prolonged release of transmitter due to a prolonged Ca^{2+} sensitivity of the presynaptic terminal after each nerve stimulus. The cause of this is probably accumulation of Na^+ inside the nerve terminal.**

At many neuromuscular junctions (nmjs) the amount of transmitter released in response to a nerve stimulus may be increased by previous impulses. This is called facilitation and involves at least two processes, each with a characteristic time course of development and of decay (1, 2). Short-term facilitation denotes the growth of the synaptic potential as the nerve is stimulated again within a few hundred milliseconds of a previous impulse (3). If stimulation at sufficiently high frequency is continued for a long time, there is further growth of the synaptic potential; a test stimulus will show enhanced release tens to hundreds of seconds after the repetitive stimulation has been stopped (4). This process at the crustacean nmj has been called "long-term facilitation" (5) and, at the vertebrate nmj (2) and certain invertebrate synapses (6), "potentiation."

Short-term facilitation involves an increase in the average number of packets of transmitter released per impulse from the nerve terminal (i.e., the quantal content) in both vertebrate (7) and invertebrate (8) nmjs. Potentiation at the vertebrate nmj also entails an increase of the quantal content (9). Both processes at the vertebrate nmj require Ca^{2+} in the bathing medium (10–12). On the other hand, long-term facilitation at the crustacean nmj seems to require external Na^+ (13, 14). While stressing that we do not know the extent to which the long-term effects in *Drosophila* and in other species resemble each other, we have adopted the terms "short-term facilitation" and "long-term facilitation" in our report on the *Drosophila* nmj. In this paper, we describe the use of single gene mutations in dissociating the underlying mechanisms.

MATERIALS AND METHODS

The Mutant. "Normal" *Drosophila melanogaster* is the Canton-Special (C-S) wild type. This strain, and a mutant isolated from it by Marilyn Woo called *bang-sensitive, bas*^{MW1}, are from S. Benzer's collection at the California Institute of Technology. The behavior of some *bang-sensitive* mutants has been described before (15). When *bas*^{MW1} flies suffer a sudden jolt caused by banging the culture vial, they become immobilized for 1–2 min, whereas normal flies are only slightly disturbed by the same treatment. This behavior was mapped by us to between *g* (44.4) and *sd* (51.5) on the X chromosome. Deletions and duplications of the X chromosome with known breakpoints in this region were used to localize *bas*^{MW1} to between 13F and 14D of the salivary gland chromosome map (16). The *bas*^{MW1} mutant shows abnormal synaptic transmission at the larval nmj (described below). Both the behavioral and the physiological abnormalities are recessive and segregate together genetically. The map position of *bas*^{MW1} is close to that of a *bang-sensitive* mutant, *bas*, isolated previously by Grigliatti *et al.* (17). Although *bas* larvae show essentially normal neuromuscular transmission, *bas/bas*^{MW1} larvae show abnormal synaptic transmission similar to that of *bas*^{MW1} larvae. Therefore these two mutants are probably allelic.

Physiological Experiments. The normal *Drosophila* larval nmj has been described (18–20). Late third instar larvae were pinned out in saline and cut open along the dorsal midline. The nerves were cut near the ventral ganglion, and the internal organs were removed. The body wall muscles and the nerves innervating them were viewed with Nomarski optics. The saline normally contained 128 mM NaCl, 2 mM KCl, 4 mM $MgCl_2$, 35.5 mM sucrose, and $CaCl_2$ as specified in each experiment. An ethylenediaminetetraacetic acid (EDTA)/Mg buffer system (21) was used when [Ca^{2+}] less than 0.1 mM was desired; and an ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA)/Mg buffering system (21) was used when [Ca^{2+}] less than 1 μM was desired. The solutions were buffered at pH 7.1 with 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes; Calbiochem, La Jolla, CA). Muscle membrane potentials were recorded intracellularly with 3 M KCl-filled glass micropipettes of resistance 20–40 MΩ. For calcium iontophoresis, micropipettes were filled with 0.5 M $CaCl_2$ and braking currents were applied. For extracellular recording, electrodes were filled with 1 M NaCl and broken to tip diameters of 2–5 μm. A suction electrode was used for stimulating the nerve. All experiments were done at room temperature (23–24°).

RESULTS

The appearance of the muscle fiber and the innervation pattern of *bas*^{MW1} larvae were indistinguishable from normal at the light microscope level. The spontaneous miniature excitatory junctional potentials (mejps) were similar in normal and mutant larvae in respect to size distribution (average, ~0.6 mV), rise time, and decay time. However, the mejp frequency in the

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Abbreviations: nmj, neuromuscular junction; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; mejp, miniature excitatory junctional potential; ejp, excitatory junctional potential.

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Table 1. Statistical properties of short-term facilitation in normal and mutant larvae

Exp.	Double pulses, no. (N)	Failures, no. 1st response, $n_{0,1}$	Failures, no. 2nd response, $n_{0,2}$	Quantal content 1st response, m_1	Quantal content 2nd response, m_2	Mean ejp, mV 1st response, V_1	Mean ejp, mV 2nd response, V_2	Short-term facilitation $\frac{m_2}{m_1}-1$	Short-term facilitation $\frac{V_2}{V_1}-1$
				Normal					
1	200	54	27	1.31	2.00	0.91	1.24	0.53	0.37
2	204	32	19	1.85	2.37	1.41	1.98	0.28	0.40
3	200	40	17	1.61	2.47	1.03	1.56	0.53	0.52
				Mutant					
4	200	68	27	1.08	2.00	0.82	1.33	0.85	0.62
5	200	62	43	1.17	1.54	0.95	1.34	0.31	0.41
6	162	29	10	1.72	2.79	0.87	1.42	0.62	0.63

Double pulses at 50-msec intervals were given once every 4 sec to either a normal or a mutant nerve in 0.14 mM Ca^{2+}. If short-term facilitation is entirely due to an increase in the quantal content (m), then $(m_2/m_1)-1$ should equal $(V_2/V_1)-1$, in which V_1 and V_2 are the mean ejp amplitudes of the 1st and 2nd responses, $m_1 = -\ln(n_{0,1}/N)$ and $m_2 = -\ln(n_{0,2}/N)$ are the quantal contents of the 1st and 2nd responses, $n_{0,1}$ and $n_{0,2}$ are the numbers of failures of the 1st and 2nd responses, and N is the total number of double pulses (7).

mutant was about 0.05/sec, about 20 times lower than the normal rate, 1–2/sec. At low frequencies of stimulation, the quantal content in a low (0.14 mM) Ca^{2+} medium was comparable in normal and mutant larvae (Table 1).

Normal Short-Term Facilitation in the *bas*^{MW1} Mutant. For both normal and *bas*^{MW1} larvae in a low (0.14 mM) Ca^{2+} medium, when two nerve stimuli were given within a few hundred milliseconds, the second excitatory junctional potential (ejp) was on the average larger than the first; the shorter the interpulse interval, the larger the second ejp (Fig. 1). Statistical analysis of many pairs of stimuli showed that there were fewer failures for the second pulse, and that the increment in the ejp was due to this increase in quantal content (Table 1). Thus, *Drosophila* shows a short-term facilitation of transmitter release like that of the frog (7).

Repetitive stimulation caused the ejp amplitude to rise to a plateau within 1 sec in both normal and *bas*^{MW1} larvae; the rate of rise and the plateau amplitude depended on the frequency of stimulation. This may be accounted for by short-term facilitation. For trains of stimuli, both linear and nonlinear summation of the short-term facilitory effects have been demonstrated for various vertebrate nmjs (3, 22). Because the short-term facilitory effect of each nerve stimulus lasts for only a few hundred milliseconds, the responses soon rise to a plateau. Further growth of the response after a second or more of repetitive stimulation should be due to some other process.

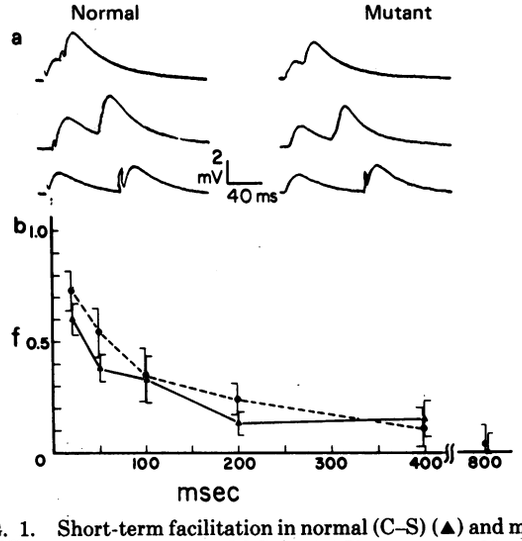

FIG. 1. Short-term facilitation in normal (C–S) (▲) and mutant (*bas*^{MW1}) (●) larvae in 0.14 mM Ca^{2+}. (*a*) Two pulses at 20-, 50-, or 100-msec intervals were given once every 10 sec; 32 responses were averaged to smooth out the quantal fluctuations. The amount of facilitation is similar for normal and mutant larvae. (*b*) Amount of short-term facilitation, f, as a function of the interval between the two pulses. f is the fractional increase of the second response above the first. Error bars indicate SD; results from four to eight experiments on different larvae are averaged for each point.

Abnormal Long-Term Facilitation in the *bas*^{MW1} Mutant. For a normal larvae in 0.14 mM Ca^{2+}, prolonged stimulation at frequencies less than 5 Hz revealed only short-term facilitation. When stimulation at frequencies greater than 5 Hz was continued, the ejp became even larger as stimulation was maintained for tens of seconds (Fig. 2). Furthermore, this process of long-term facilitation persisted for many seconds after cessation of nerve stimulation. Extracellular recordings to observe the excitatory junctional current were made at individual synaptic boutons as the nerve was stimulated at 10 Hz. These revealed, during long-term facilitation, a prolonged release of transmitter after individual motor nerve impulses. Fig. 3 shows that the duration of transmitter release increased progressively whereas the response of the postsynaptic membrane to individual quanta appeared unchanged (see also Fig. 5), indicating that long-term facilitation is presynaptic in nature. Thus, long-term facilitation differs from short-term facilitation in the time course of its development and decay, as well as in the time course of the transmitter release.

In response to a brief train of nerve stimuli, the mutant differed drastically from normal in the low Ca^{2+} (0.14 mM) medium (Fig. 4). Normal larvae displayed only short-term facilitation effects under these conditions but, after a certain point, mutant larvae produced much larger and slower ejps. These resembled those produced in normal larvae only after pro-

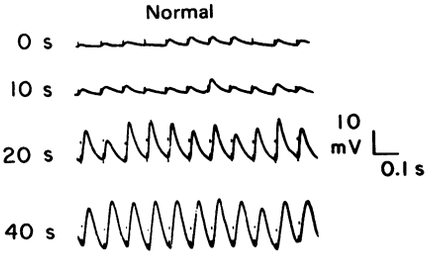

FIG. 2. ejps in the normal larvae during a train of stimuli at 10 Hz. From top to bottom: segments of records at different times (0, 10, 20, 40 sec) after the start of the train of stimuli. External Ca^{2+} concentration was 0.14 mM.

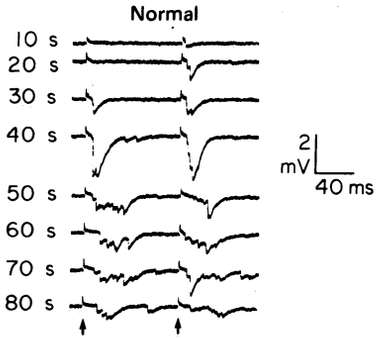

FIG. 3. Extracellular recordings of the excitatory junctional current during a train of stimuli at 10 Hz in a normal larvae in 0.14 mM Ca^{2+}. From top to bottom: segments of records (200 msec each) at 10, 20, 30, 40, 50, 60, 70, and 80 sec after the start of the train of stimuli. Note the gradual increase in duration of the excitatory junctional current. Arrows indicate stimulus artifacts.

longed stimulation. In Fig. 5 the abnormal response in a mutant larvae is compared with the effect of long-term facilitation in a normal larva. The normal nerve required stimulation for tens of seconds at 10 Hz before it gave rise to the large, prolonged release. In contrast, the mutant nerve needed only several seconds of 4 Hz stimulation to produce a similar effect. Once the prolonged release was induced by repetitive stimulation, it remained prolonged provided the frequency of test pulses given after the repetitive stimulation exceeded 4 Hz for a normal nerve, compared to 1 Hz for a mutant nerve. As shown in the following sections, various treatments that altered long-term facilitation in the normal larva altered the abnormal response of the mutant in a corresponding way. The abnormality of the mutant appears to be an unusually fast development and slow decay of long-term facilitation.

Ca^{2+} Sensitivity at Nerve Terminals during Long-Term Facilitation. The prolonged transmitter release during long-term facilitation could result from a prolonged Ca^{2+} sensitivity at the nerve terminal after arrival of the impulse. To test this possibility, we used the experimental design of Katz and Miledi (23). In 0.01 mM Ca^{2+}, brief calcium pulses were applied iontophoretically to the nerve terminal at varying time intervals before or after nerve stimulation. Under these conditions, neither nerve stimulation alone nor calcium pulses alone produced ejps. At low frequencies of stimulation, calcium pulses preceding a nerve stimulus by less than 100 msec produced transmitter release, whereas calcium pulses more than 2 msec after a nerve stimulus were ineffective, as seen in the frog (23). This was true for both normal and mutant larvae (see Fig. 6 for

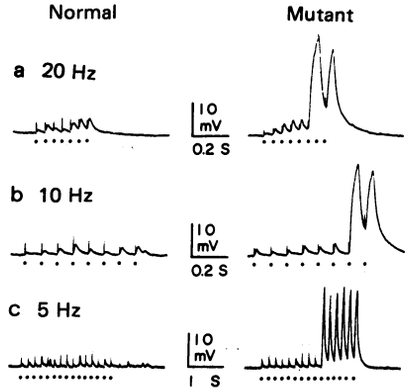

FIG. 4. ejps in normal (C–S) and mutant (bas^{MW1}) larvae during trains of stimuli at 20 Hz (*a*), 10 Hz (*b*), and 5 Hz (*c*). Dots indicate stimulus artifacts. External Ca^{2+} concentration was 0.14 mM.

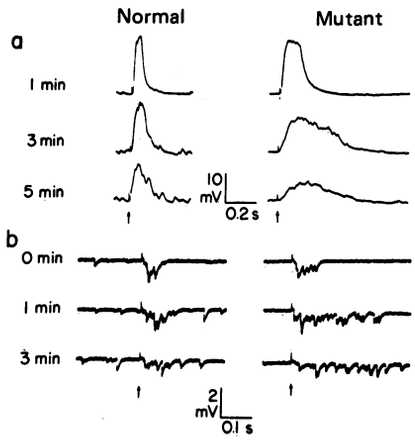

FIG. 5. Long-term facilitation in normal (C–S) and mutant (bas^{MW1}) larvae in 0.14 mM Ca^{2+}. To induce the facilitated state, the normal larval nerve was first stimulated at 10 Hz for 60 sec and then at 4 Hz for the time indicated. The mutant nerve was first stimulated at 4 Hz for 10 sec, then at 1 Hz for the time indicated. (*a*) Intracellular recordings of the ejp. (*b*) Extracellular recordings of the excitatory junctional current. Arrows indicate stimulus artifacts.

mutant and figure 5 of ref. 20 for normal). After long-term facilitation had been induced in the mutant by a 4-Hz stimulation, it could be maintained by test pulses given at 1 Hz; and a calcium pulse by itself, as late as a few hundred milliseconds after a nerve stimulus, still induced transmitter release (Fig. 6). This shows that after repetitive stimulation the release mechanism of the nerve terminal remains sensitive to calcium for a few hundred milliseconds after each nerve stimulus. In normal larvae, stimulation at about 20 Hz was required to induce long-term facilitation and 5-Hz stimulation was needed to sustain it. It was found that calcium pulses 50 msec after nerve stimulation were effective in causing transmitter release, indicating that, in both mutant and normal larvae, prolonged

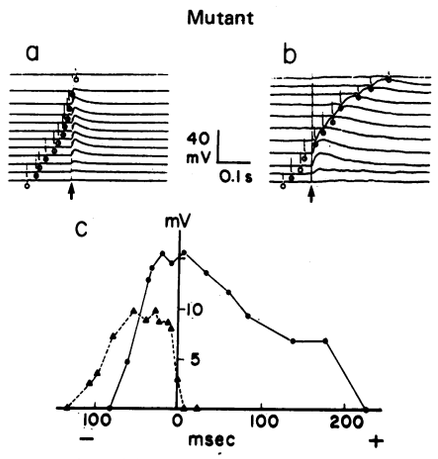

FIG. 6. ejps induced by calcium iontophoresis at various times with respect to nerve stimulation: mutant (bas^{MW1}) nerve terminal in 0.01 mM Ca^{2+}. (*a*) Unfacilitated state. The nerve was stimulated once every second. (*b*) Facilitated state after a train (about 30 sec) of 4-Hz stimulation. The nerve was stimulated once every second after the train of 4-Hz stimulation. Arrow indicates the time of nerve stimulation and circle indicates the time of the calcium pulse. (*c*) ejp amplitude as function of time interval. Nerve stimulation at time 0. At right, calcium pulse follows nerve stimulation; at left it precedes. ▲, Before 4-Hz stimulation; ●, after 4-Hz stimulation. Note that, even though the calcium pulses used before 4-Hz stimulation were larger than those used after 4-Hz stimulation, the responses were larger after 4-Hz stimulation, indicating that the nerve terminal was more sensitive to Ca^{2+} in the facilitated state.

Ca^{2+} sensitivity is responsible for the prolonged and asynchronous transmitter release during long-term facilitation.

Ca^{2+} Is Not Required for Long-Term Facilitation. Stimulation of a mutant nerve at 4 Hz in a medium containing 1 mM EGTA/4 mM Mg^{2+} and no added Ca^{2+} still produced small but prolonged ejps, as evidenced by an increase in the number of quanta released per sec; once initiated, long-term facilitation could be maintained as long as the mutant nerve was given test pulses at 1 Hz. If the bathing fluid was changed to 0.14 mM Ca^{2+} while the mutant nerve was stimulated at 1 Hz, a large, slow ejp characteristic of long-term facilitation (e.g., Fig. 5) was observed after each nerve stimulus. Stimulating the mutant nerve at 1 Hz without previous 4-Hz stimulations in the EGTA medium did not produce the large, slow ejp as the external [Ca^{2+}] was raised to 0.14 mM. Therefore, long-term facilitation developed in the mutant during 4-Hz stimulation in the EGTA medium, indicating that Ca^{2+} is not required.

A similar experiment with normal larvae required prolonged 10-Hz stimulation. This increased the mejp frequency to over 200/sec in a medium containing Ca^{2+}, 0.001 mM or less as seen in the frog (24). This elevated level of mejp persisted for several minutes after the 10-Hz stimulation, making the experiment and its interpretation difficult. However, by using a medium containing 4 mM Mg^{2+} without added Ca^{2+}, 10-Hz stimulation could be applied without inducing such an increase of the mejp frequency. Under these conditions, stimulating a normal nerve at 10 Hz prior to a 4-Hz stimulation and a concurrent increase of external [Ca^{2+}] to 0.14 mM did produce the large, slow ejp characteristic of long-term facilitation. Also, as described before, stimulating a normal nerve at high frequency in 0.01 mM Ca^{2+} appeared to prolong the Ca^{2+} sensitivity. Therefore, in both normal and mutant larvae, Ca^{2+} is probably not necessary for long-term facilitation.

Higher external [Ca^{2+}] hampered onset of long-term facilitation, requiring longer trains of stimulation at 10 Hz. Thus, in the mutant, it took about 10 stimuli at 10 Hz to induce long-term facilitation in 0.14 mM Ca^{2+} (Fig. 4), about 40 stimuli in 0.4 mM Ca^{2+}, and about 400 stimuli in 0.8 mM Ca^{2+}. Similarly, in the normal larvae, 10-Hz stimulation induced long-term facilitation in 0.14 mM Ca^{2+} but failed to do so in 0.2 mM Ca^{2+}.

Na^+ Dependence of Long-Term Facilitation. Tetrodotoxin at 1 μg/ml blocked nerve conduction in both normal and mutant larvae. However, transmitter release could still be evoked by passing depolarizing currents of 0.5- to 2-msec duration through the stimulating suction electrode. The effectiveness of such stimuli varied inversely with the nerve length between the suction electrode and the terminal, and the quantal content in low Ca^{2+} (0.1–0.2 mM) varied with the duration and amplitude of the depolarizing current pulse (20). In this situation, short-term facilitation and occasionally depression of transmitter release could be observed in both normal and mutant larvae. But long-term facilitation could no longer be produced by prolonged, high-frequency depolarizing pulses; the ejp quickly rose to a plateau due to short-term facilitation but remained constant thereafter (Fig. 7).

Nerve conduction could also be blocked by replacing external NaCl completely with sucrose. By depolarizing the nerve where it entered the muscle, we could again demonstrate short-term facilitation but not long-term facilitation in both normal and mutant larvae. Therefore, short-term facilitation does not require external Na^+ or action potentials. Long-term facilitation, on the other hand, develops only if there is a high frequency of nerve action potentials.

If the external NaCl was partially replaced with sucrose, the threshold for nerve stimulation did not change, nor did the quantal content in 0.14 mM Ca^{2+}. But the development of

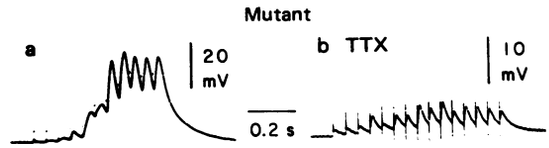

FIG. 7. ejps in a mutant (bas^{MW1}) larva before (*a*) and 5 min after (*b*) tetrodotoxin (1 μg/ml) was introduced to the bath containing 0.14 mM Ca^{2+}. The frequency of stimulation was 20 Hz. After treatment, the terminal may be depolarized electrotonically to cause transmitter release. Under these conditions, short-term facilitation persisted but long-term facilitation was never observed, even after several minutes of repetitive stimulation.

long-term facilitation was impeded. In 128 mM Na^+, stimulating a mutant nerve at 4 Hz or 10 Hz induced long-term facilitation. As the external [Na^+] was decreased to 32 mM, the number of stimuli at either frequency required for long-term facilitation increased by a factor of 2 to 4. At 8 mM Na^+, an even longer train of 10-Hz stimulation was necessary, and 4-Hz stimulation was no longer sufficient for long-term facilitation. Correspondingly, stimulating a normal nerve at 10 Hz was sufficient to cause long-term facilitation in 128 mM Na^+ but not in 32 mM Na^+.

Effect of Ouabain or Li^+ on Long-Term Facilitation. The results described so far are compatible with the hypothesis that long-term facilitation is caused by insufficient sodium pump activity, which leads to accumulation of Na^+ within terminals and accumulation of K^+ in the extracellular spaces (25, 26) because of a high frequency of nerve action potentials. This hypothesis was tested by using ouabain, a sodium pump inhibitor (27). In normal larvae without ouabain, in 0.14 mM Ca^{2+}, test pulses were applied at 1 Hz after long-term facilitation was induced by a 10 Hz stimulation. The first several test pulses elicited a large, slow ejp; then long-term facilitation decayed and the small, unfacilitated ejp was produced. This was repeated 30 min after application of 20 μM ouabain. In that case, after long-term facilitation was initiated by a 10-Hz stimulation, the large, slow, facilitated ejp could be evoked as long as test pulses were continued at 1 Hz. Similarly, in a mutant without ouabain, long-term facilitation developed during a 2-Hz stimulation and could be maintained thereafter by test pulses given once every 2 sec. With 20 μM ouabain, long-term facilitation could be induced by a 1-Hz stimulation and persisted thereafter as test pulses were given once every 5 sec. Therefore, ouabain enhances long-term facilitation in both normal and mutant larvae.

Replacing external Na^+ with Li^+ decreases the sodium pump activity, because internal Li^+ does not activate the pump (27). For a normal larva in 0.14 mM Ca^{2+}, within 15 min after replacement of half of the external Na^+ (64 mM) with Li^+, the long-term facilitation was similar to that of a mutant larva in a normal Na^+ medium. After 30 min, single nerve stimuli produced the large, slow ejps, and many spontaneous ejps were seen, presumably caused by a depolarization of the nerve terminal. For the mutant in a medium containing 0.14 mM Ca^{2+}, 64 mM Li^+, and 64 mM Na^+, within 15 min the large, slow ejps appeared spontaneously as well as after each nerve stimulus. The effect of Li^+ was reversible for normal and mutant larvae, as judged from the state of long-term facilitation 60 min after the bath was changed to a normal Na^+ medium. In summary, replacing external Na^+ with Li^+ also enhances the effect of long-term facilitation in both normal and mutant larvae.

K^+ Dependence of Long-Term Facilitation. In many organisms the activity of the sodium pump requires both external K^+ and internal Na^+ (27). If long-term facilitation is due to accumulation of Na^+ that results from a decreased activity of

the sodium pump, removing external K^+ should enhance long-term facilitation. This was found to be true. For normal larvae in a K^+-free medium containing 0.14 mM Ca^{2+}, long-term facilitation was induced by a shorter (~10 sec) train of 10-Hz stimulation and persisted indefinitely thereafter for test pulses given once per sec. For the mutant in a K^+-free medium containing 0.14 mM Ca^{2+}, long-term facilitation was initiated by a shorter (~5 sec) train of 2-Hz stimulation and persisted for many minutes for test pulses given once per 30 sec.

Increasing the external K^+ concentration accelerates sodium pump activity in mammalian nonmyelinated nerve fibers (28). If this applies to *Drosophila*, there should be less accumulation of internal Na^+ in a high K^+ medium. In the narrow space between axons and glial cells, however, the K^+ concentration is probably higher in a high K^+ medium, in spite of the higher sodium pump activity (26). We found that, in 2 mM K^+, stimulating a normal nerve at 10 or 20 Hz induced long-term facilitation; in 8 mM K^+ it did not. Similarly, for a mutant larva in 2 mM K^+, long-term facilitation could be induced by 2- or 4-Hz stimulation, whereas in 32 or 16 mM K^+, it could be induced by 4-Hz but not by 2-Hz stimulation. Thus, in both normal and mutant larvae, long-term facilitation was diminished by a high K^+ medium, suggesting that long-term facilitation is probably not caused by accumulation of extracellular K^+ during repetitive stimulation but rather by an increase in the intracellular Na^+ concentration.

DISCUSSION

The existence of a mutant with abnormal long-term facilitation but normal short-term facilitation suggests that, in *Drosophila*, these two processes have different underlying mechanisms. This is further supported by the finding that removing external Na^+ or adding tetrodotoxin to the bath eliminated long-term facilitation but not short-term facilitation. Short-term facilitation in *Drosophila* is similar to that at the frog nmj in magnitude and time course. By analogy, it is probably caused by some action of Ca^{2+}, as has been shown in the frog by Katz and Miledi (10). This possibility is supported by the finding that short-term facilitation occurs in a Na^+-free medium containing only K^+, Ca^{2+}, Mg^{2+}, and Cl^-. Long-term facilitation, on the other hand, can be induced in low external [Ca^{2+}] and appears to be caused by Na^+ accumulation inside the nerve terminal because: (*i*) it is eliminated if nerve conduction is blocked by tetrodotoxin or by removing external Na^+, (*ii*) decreasing external Na^+ impedes its development without blocking nerve conduction, (*iii*) removing external K^+ or replacing external Na^+ with Li^+ greatly enhances it (these processes presumably decrease sodium pump activity), (*iv*) ouabain enhances it, and (*v*) increasing external K^+ diminishes it.

In *Drosophila*, long-term facilitation is found to be associated with a prolonged Ca^{2+} sensitivity at the nerve terminal and, consequently, prolonged release of transmitter. How an accumulation of internal Na^+ prolongs the presynaptic Ca^{2+} sensitivity is unknown. It should be stressed that our measurement of the Ca^{2+} sensitivity may include presynaptic events other than the opening of calcium channels. For example, Na^+ accumulation inside the terminal might initiate a voltage-dependent Na^+–Ca^{2+} exchange mechanism (29) to transport Ca^{2+} into the terminal in exchange for Na^+. This would allow Ca^{2+}, iontophoretically applied after the nerve stimulus, to enter the terminal and cause transmitter release.

Long-term facilitation in the bas^{MW1} mutant shares all the properties mentioned above with that in normal larvae, but it develops much faster and decays much more slowly. We do not know the cause of this abnormality. Conceivably, it could be a defect in the sodium pump or an enhanced Na^+ sensitivity of the machinery responsible for long-term facilitation. The relationship of the behavioral abnormality to the abnormal long-term facilitation, if any, is also unknown. The presynaptic nature of this mutant's defect is consistent with the findings by J. S. Feitelson and L. M. Hall (personal communication), who used the mosaic fate-mapping method to show that for *bas*, which is probably an allele of bas^{MW1}, the primary focus of its defect is in the nervous system rather than in the muscle.

We thank Dr. Seymour Benzer for advice, equipment, and mutant. We appreciate the helpful criticisms of this manuscript by many, especially Drs. Seymour Benzer, Stephen Kuffler, Michael Dennis, A. R. Martin, and William Harris. This work was supported in part by Grant GB-27228 from the National Science Foundation to Seymour Benzer, a postdoctoral fellowship from the Muscular Dystrophy Association to Y.N.J., and a postdoctoral fellowship from the National Institutes of Health to L.Y.J.

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