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Synaptic Plasticity in *Drosophila* Memory and Hyperexcitable Mutants: Role of cAMP Cascade

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Activity-dependent synaptic plasticity has been implicated in the refinement and modification of neural circuits during development and learning. Previous studies show that activity-induced facilitation and potentiation are disrupted at larval neuromuscular junctions in the memory mutants *dunce* (*dnc*) and *rutabaga* (*rut*) of *Drosophila*. The diminished learning–memory capacity and synaptic transmission plasticity have been associated with altered cAMP levels since *dnc* affects the cAMP-specific phosphodiesterase and *rut* affects adenylate cyclase. In this study, the morphology of larval motor axon terminals was examined by anti-HRP immunohistochemistry. It was found that the numbers of terminal varicosities and branches were increased in *dnc* mutants, which have elevated cAMP concentrations. Such increase was suppressed in *dnc rut* double mutants by *rut* mutations, which reduce cAMP synthesis. More profuse projections of larval motor axons have also been reported in double-mutant combinations of *ether à go-go* (*eag*) and *Shaker* (*Sh*) alleles, which display greatly enhanced nerve activity as a result of reduction in different K^+ currents. Therefore, we examined combinations of *dnc* and *rut* with *eag* and *Sh* mutations to explore the possible relation between activity- and cAMP-induced morphological changes. We found that the expanded projections in *dnc* were further enhanced in double mutants of *dnc* with either *eag* or *Sh*, an effect that could again be suppressed by *rut*. The results provide evidence for altered plasticity of synaptic morphology in memory mutants *dnc* and *rut* and suggest a role of cAMP cascade in mediating activity-dependent synaptic plasticity.

Increasing evidence has suggested that electric activity–controlled mechanisms may play a crucial role in the "fine tuning" of functional architecture of neural circuits (Harris, 1981; Udin and Fawcett, 1988; Lnenicka and Murphey, 1989; Constantine-Paton et al., 1990). One such classical example is the role of the visual experience in shaping synaptic patterns of the visual cortex (Hubel et al., 1977). More direct evidence has been revealed by application of TTX to block the Na^+ channel-dependent action potential (Brown and Ironton, 1977; Harris, 1981; Meyer, 1982; Schmidt and Edwards, 1983; Reh and Constantine-Paton, 1985). TTX blockade of retinal ganglion cells prevents the formation of ocular dominance stripes that delineate the projections from the normal and ectopic eyes in the frog tectum (Reh and Constantine-Paton, 1985). TTX blockade also induces motor nerve terminal sprouting in vertebrate neuromuscular junctions (Brown and Ironton, 1977).

In invertebrates, electric activity has also been shown to be involved in the regulation of neural circuits during development (Lnenicka and Murphey, 1989; Atwood and Govind, 1990). Chronic stimulation of relatively silent phasic motoneurons in the crayfish induces changes in the number and shape of synapses formed at the motor terminals (Lnenicka et al., 1986). Hyperexcitability induced by genetic lesions of K^+ channels also correlates to expanded terminal projection in *Drosophila* motor axons (Budnik et al., 1990). In addition, direct electric stimulation on cultured neurons has been reported to influence growth cone motility and neurite elongation (Kater et al., 1988; Kater and Mills, 1991).

The molecular basis of this electric activity–controlled mechanism remains to be established. The activation of the NMDA subclass of excitatory glutamate receptors has been suggested to mediate the activity-dependent plasticity in the visual cortex and in the retinotectal projection (Constantine-Paton et al., 1990; Cline, 1991), but there is no unambiguous information on the biochemical events subsequent to NMDA receptor activation. Even less is known in other preparations. Studies of cultured neurons have provided suggestive clues. For instance, changes in intracellular Ca^{2+} or cAMP concentrations have been shown to be critical for regulating the growth cone motility and neurite elongation (Forscher et al., 1987; Kater et al., 1988; Kater and Mills, 1991). It appears that second messenger cascades may be important factors in shaping nerve terminal arborization.

We examined *Drosophila* mutants with altered cAMP cascade to investigate whether the cAMP cascade is involved in modifying the nerve terminal arborization and how the pathway is related to activity-dependent synaptic plasticity. The *dunce* (*dnc*) and *rutabaga* (*rut*) mutants, initially isolated as learning and memory mutants, have been shown to be defective in the cAMP metabolic pathway (Tully, 1984; Dudai, 1988). The *dnc* gene encodes phosphodiesterase II, an enzyme for cAMP hydrolysis (Byers et al., 1981; Chen et al., 1986). The reduction (dnc^1) or elimination (dnc^{M11} and dnc^{M14}) of phosphodiesterase II activity cause elevated cAMP in the *dnc* alleles (Byers et al., 1981). In contrast, the *rut* locus appears to code for a subunit of adenylate cyclase (Livingstone et al., 1984; Krupinski et al., 1989). The rut^1 mutation eliminates Ca^{2+}/calmodulin (Ca^{2+}/CaM) activation of adenylate cyclase and reduces its basal activity (Dudai and Zvi, 1984; Livingstone et al., 1984), leading to reduced cAMP concentration. A weaker allele, rut^2, only reduces the

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basal activity of adenylate cyclase, but does not abolish its activation by Ca^{2+}/CaM (Feany, 1990).

We report that *dnc* mutants showed an enhanced motor terminal arborization, suggesting that cAMP levels influence the size of motor axon projections. This was further supported by the result that the effect of *dnc* mutations on nerve terminal arborization could be counterbalanced by rut^1 as reflected in *dnc* rut^1 double mutants.

It has been reported that hyperexcitability observed in double mutants with defects in different K^+ channels or with abnormal expression of Na^+ channels can be correlated with expansion of terminal projections (Budnik et al., 1990). For example, enhanced arborization can be seen in double-mutant combinations of *ether à go-go* (*eag*) and *Shaker* (*Sh*), each affecting different K^+ channels (Jan et al., 1977; Wu et al., 1983; Ganetzky and Wu, 1986; Salkoff and Tanouye, 1986; Zhong and Wu, 1991b). Such effect is not present in *eag* or *Sh* single mutants and can be suppressed in *eag Sh*; *nap* (*no action potential*) triple mutants (Budnik et al., 1990) by *nap*, a mutation causing reduction in Na^+ channel density (Wu et al., 1978; Jackson et al., 1984; Ganetzky and Wu, 1986). We examined the combinations of *dnc* and *rut* with hyperexcitability mutations and found that *dnc* mutations potentiated the effects of either *Sh* or *eag* single mutants. The enhancement could again be counterbalanced by the presence of rut^1 in *dnc rut Sh* triple mutants. However, the effect of the extreme hyperexcitability in *eag Sh* was not further promoted in *dnc eag Sh*. Our results indicated that the cAMP cascade may be crucial to regulation of nerve terminal arborization, and support the view that synaptic plasticity may be mediated by activity-dependent accumulation of intracellular Ca^{2+} that in turn triggers second messenger cascades.

Materials and Methods

Fly stocks

Drosophila melanogaster stocks were reared at room temperature (20–23°C). The Canton-Special strain was used as normal control flies. The molecular lesions and physiological characteristics of the mutants used are summarized below.

Sh^{KO120}. The *Shaker* locus (mapped to 1–57.4) encodes K^+-channel subunits. This *Sh* allele displays reduced A-type K^+ current (I_A) in larval muscles and enhances nerve excitability and synaptic transmission at larval neuromuscular junctions (Ganetzky and Wu, 1983; Haugland and Wu, 1990).

Sh^{133}. The mutation eliminates I_A in larval muscles and enhancing transmission at larval neuromuscular junctions (Jan et al., 1977; Ganetzky and Wu, 1983; Haugland and Wu, 1990).

eag^1. The *ether à go-go* locus (1–48.9) encodes a K^+-channel polypeptide (Warmke et al., 1991). The mutation reduces several K^+ currents in larval muscles and interacts synergistically with *Sh* mutations to enhance nerve excitability and neuromuscular transmission (Ganetzky and Wu, 1983; Wu et al., 1983; Zhong and Wu, 1991b).

dnc^1. This allele of *dunce* (1–4.6) was isolated as a learning mutant (Dudai et al., 1976). The mutation reduces the phosphodiesterase II activity, leading to a higher concentration of cAMP (Byers et al., 1981).

dnc^{M14}. This extreme allele was isolated because of female sterility (Mohler, 1977). It also eliminates phosphodiesterase II activity and shows learning deficiency (Byers et al., 1981).

dnc^{M11}. The phenotype of this mutant is similar to dnc^{M11} (Mohler, 1977; Byers et al., 1981). In addition, protein kinase C activity is reported to be altered in this stock by a separate mutation at a closely linked locus yet to be identified (Devay et al., 1989).

rut^1. This allele of *rutabaga* (1–46) was isolated by deficiency in an associative learning paradigm (Aceves-Pina et al., 1983). The mutation eliminates Ca^{2+}/CaM activation of adenylate cyclase and reduces its basal activity (Dudai and Zvi, 1984; Livingstone et al., 1984).

rut^2. This allele was isolated by its partial suppression of female sterility in dnc^{M11} and dnc^{M14} (Bellen et al., 1987). The basal activity of adenylate cyclase is reduced but its activation by Ca^{2+}/CaM is preserved (Feany, 1990).

The double- and triple-mutant strains used were derived from recombinations among those described above. The presence of *eag, Sh, dnc,* and *rut* mutations in the recombinants was confirmed by testing the leg-shaking behavior (Ganetzky and Wu, 1986), wing position (Ganetzky and Wu, 1983; Drysdale et al., 1991), *dnc*-associated female sterility (Mohler, 1977), and rescue of this sterility by *rut* (Livingstone et al., 1984; Bellen et al., 1987), as well as flanking morphological markers. The morphological markers used included *y, cv, v, g, sd,* and *f,* which have been described in Lindsley and Grell (1968).

Immunohistochemistry

The larval neuromuscular preparation and the anti-HRP staining protocol have been described previously (Johansen et al., 1989a; Budnik et al., 1990). Briefly, late third instar larvae were dissected in saline and then fixed in nonalcoholic Bouin's solution (25 ml formalin, 5 ml glacial acetic acid, 75 ml saturated picric acid) for 1–2 hr. The samples were then incubated sequentially with 1:200 anti-HRP (Sigma) and 1:20 HRP-conjugated goat anti-rabbit IgG (Cappel). Staining was revealed by HRP-catalyzed diaminobenzidine reaction.

Results

Increased terminal branching and varicosities in dnc *mutants*

The segmental body-wall muscle fibers in *Drosophila* larvae are organized in a regular pattern (Crossley, 1978; Johansen et al., 1989a). Within individual identifiable fibers, motor axon terminals exhibit distinct branching patterns (Johansen et al., 1989a,b; Budnik et al., 1990). Our studies focused on muscle fibers 12 and 13 in abdominal segment 3 of third instar larvae (Johansen et al., 1989a; Budnik et al., 1990). The motor terminals at these two muscle fibers have been extensively studied with anti-HRP immunohistochemical staining (Budnik et al., 1990), which specifically labels insect neurons (Jan and Jan, 1982). Nerve processes on these two muscle fibers project both anteriorly and posteriorly with varicosities distributed along their branches (Fig. 1; see also Johansen et al., 1989a; Budnik et al., 1990). Such varicosities are thought to be synaptic sites (Bailey and Chen, 1983; Johansen et al., 1989a; Budnik et al., 1990).

Using the same immunohistochemical technique, we compared the numbers of varicosities and branches in normal and *dnc* larvae. It is important to examine multiple *dnc* alleles and their heterozygous combinations such that any abnormal morphology observed could be attributed to the *dnc* locus. In three independently isolated alleles, dnc^1, dnc^{M11}, and dnc^{M14}, phosphodiesterase II activity is reduced in dnc^1 and eliminated in the other two, leading to elevated cAMP and learning deficits (Byers et al., 1981; Tully, 1984; Dudai, 1988). The number of terminal varicosities was found to be increased in dnc^1 and dnc^{M14} mutants (Figs. 1, 2). Furthermore, dnc^1/dnc^{M11} (Fig. 2) heterozygote showed similar increase in the number of varicosities. However, such a change was not observed in dnc^{M11} larvae [dnc^{M11} (mean ± SD): 234 ± 29, 6 larvae; normal: 238 ± 39, 23 larvae; see Materials and Methods and Discussion for the possibility of a separate mutation on the dnc^{M11} chromosome].

The increase in varicosity number was associated with more extensive branching, as suggested by the representative tracings in Figure 1. A branch is defined as a terminal process containing at least two varicosities. The total number of all terminal branches in both muscles 12 and 13 in normal and *dnc* larvae is summarized in Figure 2, which indicates statistically significant increases in dnc^1, dnc^{M14}, and dnc^1/dnc^{M11} larvae. To illustrate the extent and variability of branching in different genotypes, we

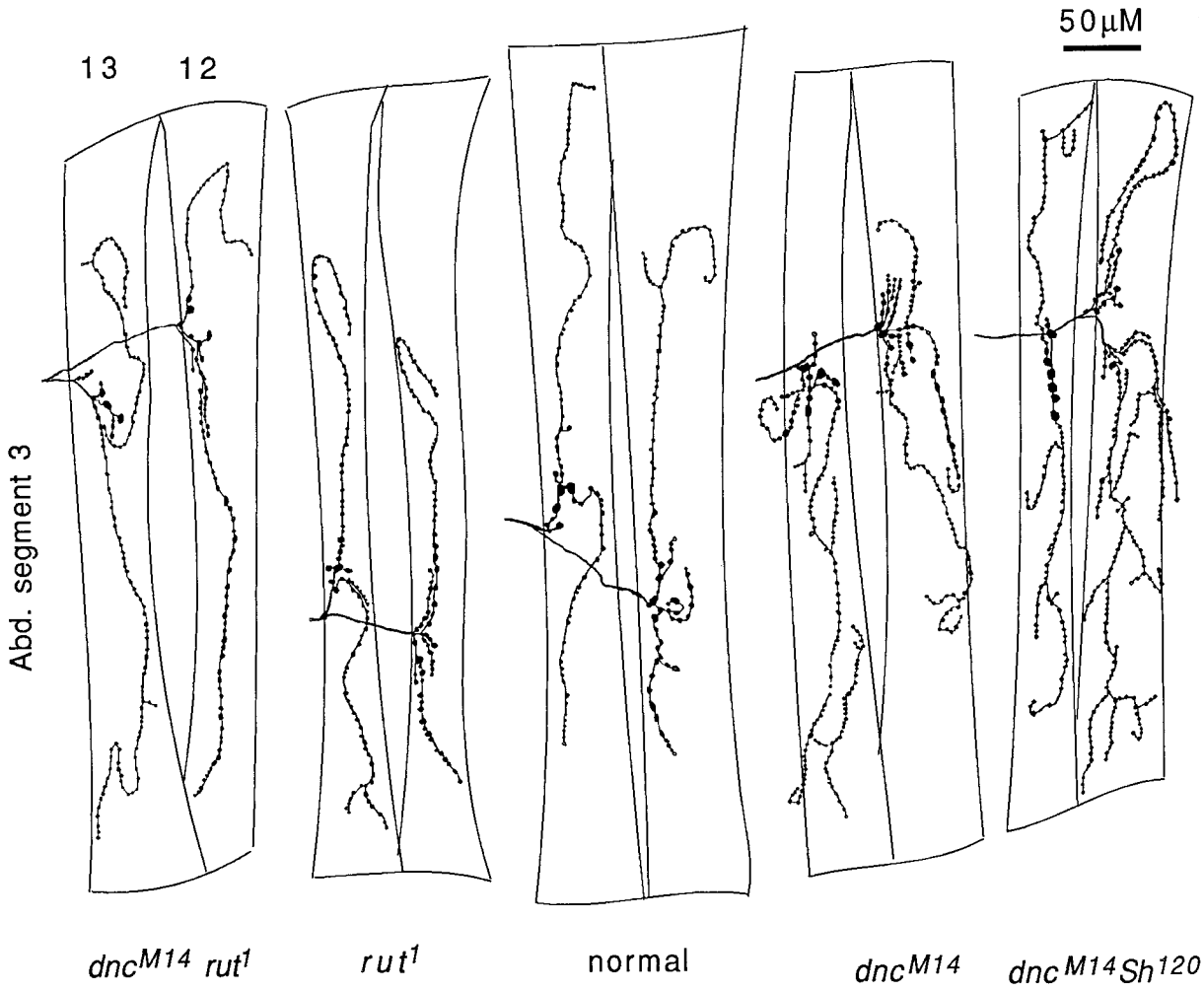

Figure 1. Camera lucida tracings of anti-HRP staining of motor axon terminals on muscle fibers 12 and 13 of abdominal segment 3 in third instar larvae of *Drosophila*. Data for normal and *rut* were from a right hemisegment (posterior on *top*), and those from $dnc^{M14}rut^1$, dnc^{M14}, and $dnc^{M14}Sh^{120}$ were from a left hemisegment (anterior on *top*). The fine neurites in the tracings presented here are thickened for clarity, partially masking some smaller varicosities. Individual varicosities were more clearly resolved in photomicrographs (see, e.g., Budnik et al., 1990).

compiled isomorphic representations (Fig. 3), each depicting the branching pattern of a single primary process that showed the highest number of branches in either muscle fiber 12 or 13 individual larvae. Branching appears to be more extensive in the *dnc* alleles as indicated by more higher-order branches (Fig. 3).

Suppression of dnc*-induced enhancement by* rut *mutations*

If the increase in branching and number of varicosities in *dnc* alleles is attributable to elevated cAMP, the alterations may be counterbalanced by the *rut* mutations. The *rut* gene is thought to encode a subunit of adenylate cyclase for cAMP synthesis (Livingstone et al., 1984; Krupinski et al., 1989; Feany, 1990). The basal cAMP synthesis is lowered in both rut^1 and rut^2 (Dudai and Zvi, 1984; Livingstone et al., 1984; Feany, 1990). However, the Ca^{2+}/CaM-dependent activation of adenylate cyclase is eliminated in rut^1 flies (Dudai and Zvi, 1984; Livingstone et al., 1984) but retained in the weaker allele rut^2 (Feany, 1990). We examined *rut* larvae as well as *dnc rut* double mutants.

The number of branches and varicosities in rut^1 showed a slight, but statistically insignificant (t test, $p > 0.05$), decrease as compared to normal larvae (Fig. 2). However, the effects of dnc^{M14} on terminal morphology were suppressed in the double mutant $dnc^{M14}rut^1$ (Figs. 1–3). Interestingly, the numbers of varicosities and branches in $dnc^{M14}rut^2$ remained similar to that in dnc^{M14} (Fig. 2), but terminal morphology returned to normal in the mutant $dnc^{M14}rut^1/dnc^{M14}rut^2$ (Fig. 2). This result is in agreement with biochemical measurements that indicate that cAMP synthesis in rut^2/rut^1 heterozygotes is lower than that in rut^2 homozygotes (Feany, 1990).

Synergistic effects of dnc *with hyperexcitability mutations*

Double mutants of different *eag* and *Sh* alleles show greatly enhanced neuronal excitability (Ganetzky and Wu, 1983) and display morphological changes in motor terminals (Budnik et al., 1990) similar to those in *dnc* alleles (Figs. 4, 5). Action potential duration is increased in *Sh* mutants as shown in the cervical giant nerve fibers (Tanouye and Ferrus, 1985), and neuromuscular transmission is also enchanced in both *eag* and *Sh* larvae (Jan et al., 1977; Ganetzky and Wu, 1983). Electrophysiological recordings from *eag Sh* double mutants have demonstrated a synergistic interaction between the two mutations, leading to abnormal spontaneous firing (Ganetzky and Wu, 1983; Budnik et al., 1990) and greatly enhanced transmitter release

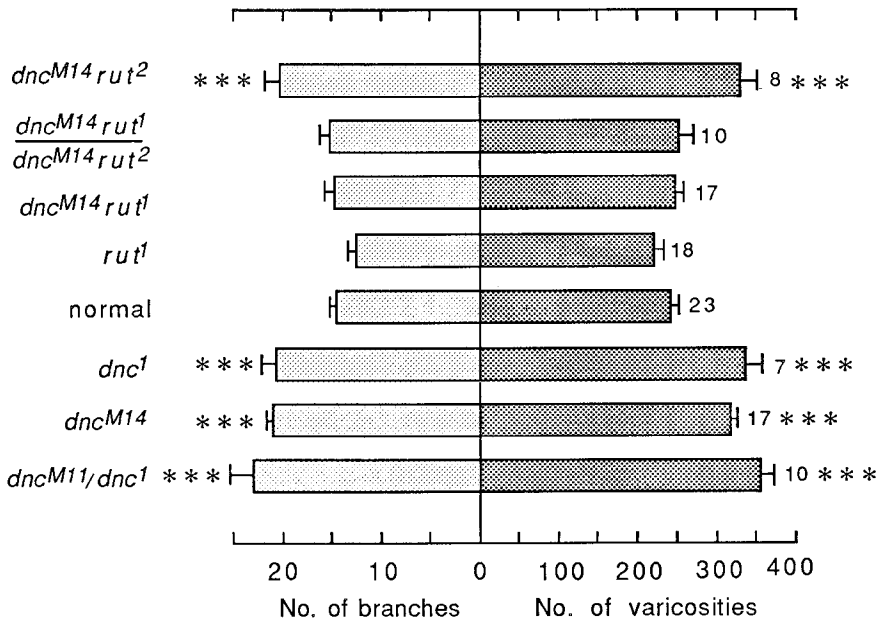

Figure 2. Increase in number of branches and varicosities in *dnc* alleles and suppression of *dnc* phenotype by *rut*: the total numbers of varicosities and branches on both muscle fibers 12 and 13 in one hemisegment. A branch is defined as a terminal process containing at least two varicosities. The mean and SEM in each genotype are presented for the number of larvae indicated. For each batch of samples, we used muscles 12 and 13 in each larva from the same side of segment 3, and only if this was prevented by damage or unsatisfactory staining was the other side used. Mutant data are compared to normal with *t* test, and statistically significant differences are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

(Ganetzky and Wu, 1983) at larval neuromuscular junctions. Combinations of *dnc* and *eag* or *Sh* provide a means to study the possible mechanistic connection between *dnc*- and *eag Sh*-induced morphological changes.

Neither *eag* nor *Sh* alone affected the morphology of axon terminals significantly (Figs. 4, 5; also see Budnik et al., 1990). However, both mutations were found to interact with the *dnc* mutation to enhance the *dnc* phenotypes. Compared to dnc^{M14} (Fig. 4), the number of varicosities was increased in double mutants $dnc^{M14}Sh^{120}$ (*t* test, $p < 0.02$) and $dnc^{M14}eag^{1}$ ($p < 0.05$) and the number of branches was increased in $dnc^{M14}Sh^{120}$ ($p < 0.02$) and $dnc^{M14}Sh^{133}$ ($p < 0.05$). The enhancement by *eag* or *Sh* was more evident in the extent of higher-order branching as indicated by the isomorphic representations (Fig. 5). The above double-mutant phenotypes could again be suppressed by rut^{1} as shown in the triple mutant $dnc^{M14}rut^{1}Sh^{120}$ (Figs. 4, 5).

Surprisingly, we observed no further increase (*t* test, $p > 0.05$) in the number of varicosities or branches in the triple mutant $dnc^{M14}eag^{1}Sh^{120}$ and $dnc^{M14}eag^{1}Sh^{133}$ compared to dnc^{M14} or *eag Sh* mutants (Fig. 4). Moreover, there was a statistically significant decrease in the number of branches compared to $dnc^{M14}Sh$ ($p < 0.02$) or $dnc^{M14}eag^{1}$ ($p < 0.05$) (see Discussion).

Discussion

cAMP-induced nerve terminal expansion

It has been shown that injecting cAMP into *Aplysia* sensory neurons increases the number of varicosities (Nazif et al., 1991). In addition, application of cAMP can influence growth cone motility (Forscher et al., 1987), neurite elongation (Rydel and Greene, 1988), and synaptogenesis (Dubinsky and Fischbach, 1990) in cultured neurons. The present study revealed increased terminal branching and synaptic contacts in multiple *dnc* alleles; these defects could be rescued by mutations at a second locus, *rut*. Since *dnc* and *rut* mutations disrupt different steps in the cAMP metabolism pathway (Byers et al., 1981; Dudai and Zvi, 1984; Livingstone et al., 1984; Tully, 1984; Dudai, 1988), our results provide *in vivo* genetic evidence that alterations in cAMP concentration affect motor terminal morphology.

Although expanded nerve terminal projection was not observed in homozygous dnc^{M11} larvae, the phenotype of dnc^{1}/dnc^{M11} heterozygotes (Figs. 2, 3) supports the idea that the dnc^{M11} chromosome used may carry some unidentified recessive mutations (Devay et al., 1989; see also Materials and Methods) that masked the *dnc* effect on terminal arbors. This recessive trait apparently manifests only in homozygous form but is not expressed in heterozygotes carrying two different *dnc* chromosomes.

The cAMP-induced nerve terminal expansion is most evident in high-order branches, which contain small-sized varicosities

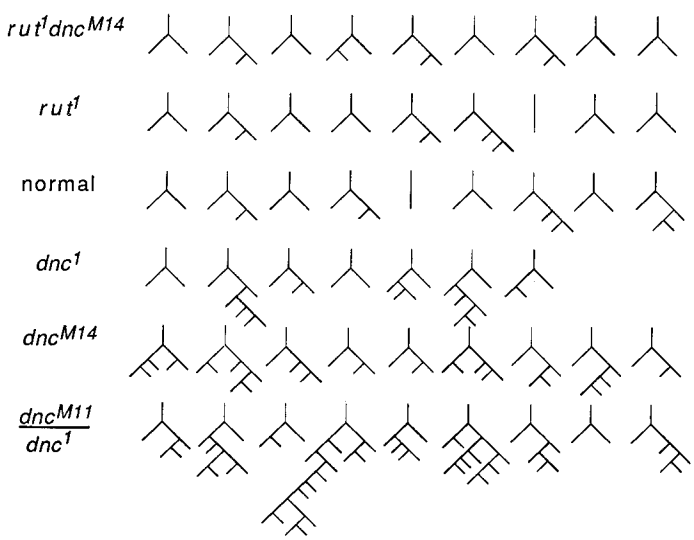

Figure 3. Isomorphic representations of branching pattern. Each representation illustrates the branching pattern of the single primary process that showed the highest number of branching in either muscle fiber 12 or 13. More extensive branching is seen in *dnc* alleles while the enhanced branching is suppressed by additional *rut* mutations in *dnc rut* double mutants. If the sample number is higher than nine in a genotype, only the first nine larvae during sampling are presented.

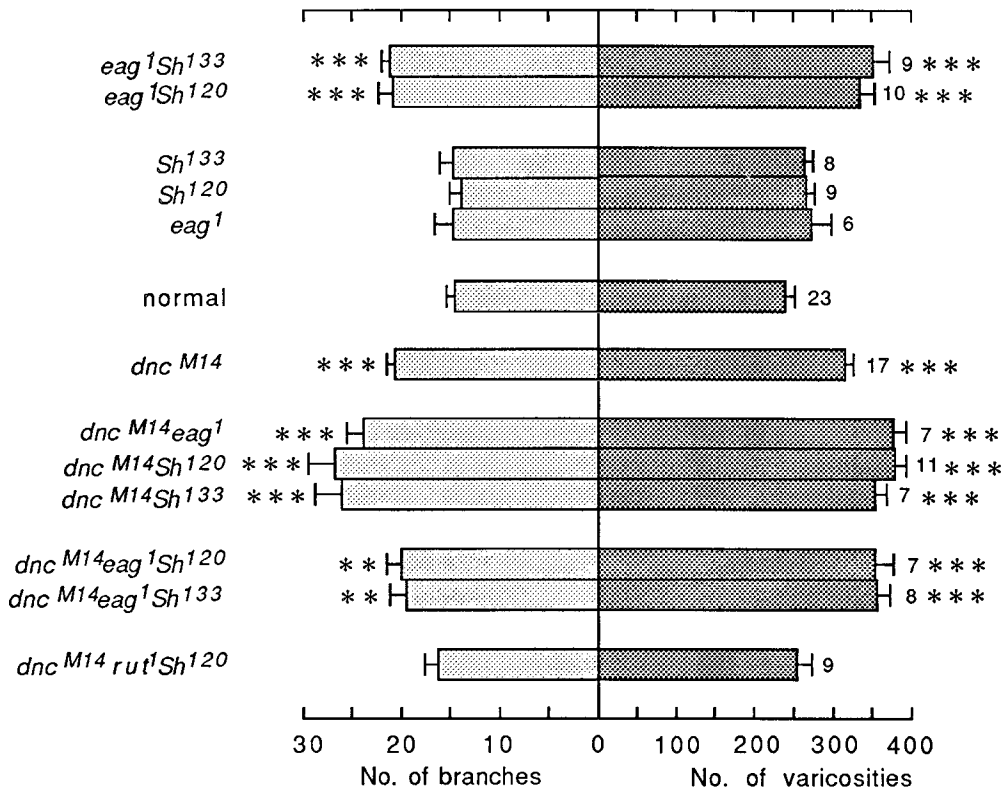

Figure 4. Changes in morphology of motor terminals induced by altered nerve excitability and cAMP levels. Data collection and analysis were the same as in Figure 2. Branches and varicosities were increased in *eag Sh* double mutants but not in either *eag* or *Sh* mutants (*eag*, *Sh*, and *eag Sh* data based on results shown in Budnik et al., 1990, plus new samples). However, *eag* and *Sh* significantly enhanced the *dnc* phenotype in *dnc eag* and *dnc Sh* double mutants (see Results). This enhancement was suppressed by *rut* in the *dnc rut Sh* triple mutant.

(Figs. 1, 3, 5). It has been reported that the larval body-wall muscle fibers examined here are innervated by at least two motor neurons (Jan and Jan, 1976; Sink and Whitington, 1991). Therefore, it would be interesting to examine the possibility that varicosities of different sizes may originate from different motor neurons with distinct physiological characteristics. This information will be important to determining whether terminal expansion is differentially expressed in a motor neuron of a particular physiological type. Our current method does not allow identification of individual branches with a particular motor neuron; new techniques should be developed to resolve this problem.

Mechanism underlying activity-dependent enhancement of terminal arborization

The cAMP level has been reported to be regulated by neural activity. For instance, tetanic stimulation associated with the induction of long-term potentiation elevates cAMP concentration via Ca^{2+}/CaM-dependent adenylate cyclase in the mammalian hippocampus (Chetkovich and Sweatt, 1990). Furthermore, the increased neural activity that induces sensitization in *Aplysia* is known to stimulate cAMP synthesis in the sensory neurons by 5-HT released from the presynaptic neuron (Kandel and Schwartz, 1982). Our genetic manipulations corroborate with the above findings and suggest a possible relationship between the cAMP- and activity-induced morphological changes, as follows.

Enhanced neural activity in *eag* and *Sh* mutants may increase Ca^{2+} influx and therefore stimulate cAMP synthesis via Ca^{2+}/CaM activation of adenylate cyclase. In fact, higher phosphorylation levels have been reported in Sh^{133} (Buxbaum and Dudai, 1989). Accumulation of cAMP could be further enhanced in the double mutants *dnc eag* and *dnc Sh* because *dnc* blocks cAMP degradation. The compounding effect of *dnc* with hyperexcitability mutations thus leads to more extensive branching and increase in varicosities (Figs. 4, 5). Such effects are suppressed in the triple mutants *dnc rut*^{1} *Sh* since *rut*^{1} eliminates Ca^{2+}/CaM-activated cAMP synthesis.

We cannot rule out the possibility that terminal expansion may arise from other forms of interactions between cAMP synthesis and neuronal activity. Increased cAMP levels in *dnc* may lead to phosphorylation of ion channels that, in turn, further enhance excitability in *eag* or *Sh* mutants. However, voltage-clamp measurements in *dnc* larval muscles did not suggest that Ca^{2+} or K^+ currents are modulated in the direction that increases membrane excitability (Zhong, 1991).

Conversely, enhanced neural activity in *Sh* or *eag* may increase release of certain neurotransmitters, such as octopamine, which in turn stimulate synthesis of cAMP. In particular, octopaminergic nerve terminals have been found in larval neuromuscular junctions (Halpern et al., 1988). However, the receptor-coupled cAMP synthesis, such as octopamine-stimulated response, is reportedly near normal in *rut* mutants (Dudai et al., 1983). The fact that *rut* could rescue *dnc* or *dnc Sh* phenotypes (Figs. 2, 4) suggests that stimulated cAMP synthesis by octopamine cannot be a major factor in enhancing terminal arborization.

The results from the triple mutants *dnc eag Sh* suggest that nerve terminal may not further expand in response to increased cAMP synthesis once it is beyond a threshold or optimal level. The Ca^{2+}/CaM-activated cAMP synthesis is supposed to be even higher in the triple mutants than that in the double mutants *dnc eag* or *dnc Sh* because of the synergistic effect of *eag* and *Sh* on excitability. However, terminal arborization was slightly re-

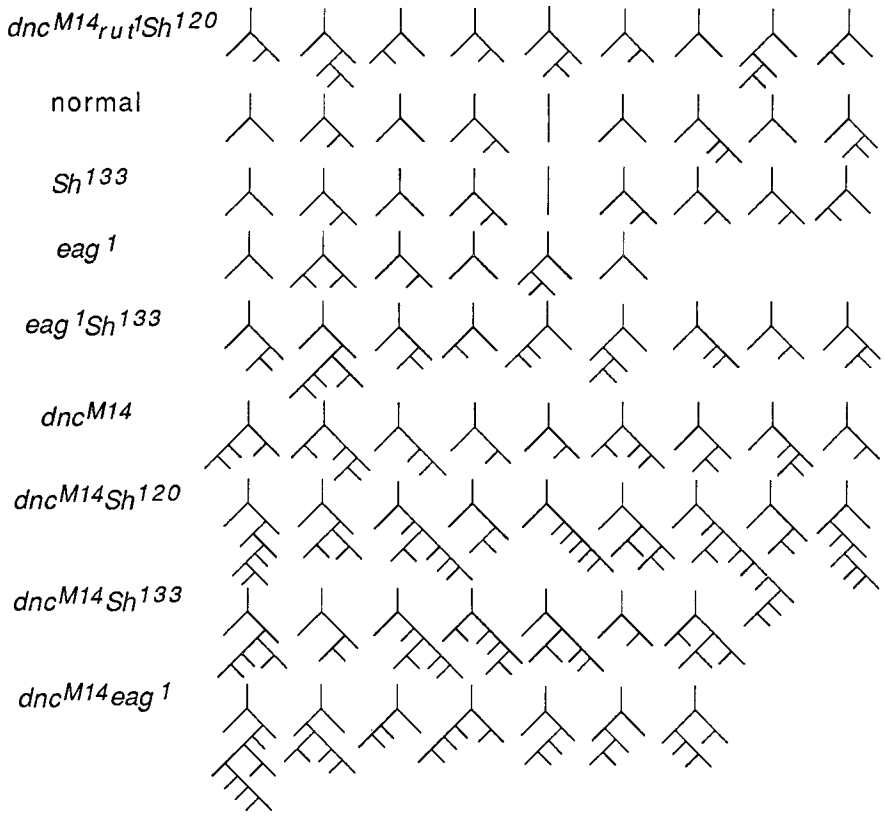

Figure 5. Isomorphic representations of branching patterns in different genotypes. Interactions between *Sh* or *eag* with *dnc* mutations induce further ramification in nerve terminal branching.

duced in *dnc eag Sh* as compared to *dnc eag* or *dnc Sh* (Fig. 4). One possibility is that if cAMP is higher than an optimal level, presumably in the case of the triple mutants *dnc eag Sh,* terminal growth may not be further promoted. This is not surprising since the operation of some steps in second messenger systems requires optimal stimuli. For example, Ca^{2+} activation of adenylate cyclase occurs only in a narrow concentration range (Livingstone et al., 1984; Feany, 1990). Other complications, such as compartmentalization of the subtypes of cAMP-dependent protein kinases with different substrates, may also induce nonlinear cellular responses (Nairn et al., 1985; Adam and Friedrich, 1988).

Alternatively, increased intracellular Ca^{2+} concentrations may also activate Ca^{2+}/CaM-dependent or Ca^{2+}/phospholipid-dependent protein kinases (Nairn et al., 1985), in addition to stimulating cAMP synthesis. These protein kinases may have different substrates or may share the same targets with cAMP-dependent protein kinase but exerting differential cellular effects (Nairn et al., 1985). Different sensitivity of these cascades to Ca^{2+} concentration may further complicate the situation. Studies of cultured snail neurons show that axonal outgrowth is promoted only in an optimal range of intracellular Ca^{2+} concentration; concentrations outside this range result in decreased outgrowth (Kater and Mills, 1991).

Altered synaptic plasticity in terminal arborization of memory mutants

In the rat and *Aplysia,* nerve terminal projections and synaptic connectivity are thought to be modified in association with learning processes (Bailey and Chen, 1983; Greenough and Bailey, 1988). The fine tuning of morphological structures may be defective across the nervous system in *Drosophila* memory mutants. It has been reported that the number of nerve fibers in the mushroom body (Balling et al., 1987) and the varicosity number in a branch of an identified sensory neuron (Corfas and Dudai, 1991) are altered in certain *dnc* and *rut* alleles. In addition, reduction in growth cone motility has been indicated in cultured neurons from the *dnc* and *rut* larval CNS (Y.-T. Kim and C.-F. Wu, unpublished observations). We have shown that *dnc* may further potentiate activity-dependent modification of nerve terminal morphology (Fig. 4). It will be important to determine how this defective regulation in morphological plasticity acts together with altered facilitation and potentiation in synaptic transmission reported in these mutants (Zhong and Wu, 1991a) to cause learning and memory deficiency.

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