cies on both islands; and *G. igata* is more common than on Hen and Little Barrier.

Changes with time on another offshore island, Cuvier, are also instructive. Until 1959 Cuvier supported cats, wild goats, and domestic stock animals, and at least five exotic bird species bred in the forest. After eradication of cats and goats and fencing of stock by 1964, a dense understory regenerated, and the four European exotics disappeared from forest, the Australian *Z. lateralis* nearly disappeared, and *G. igata* declined. These changes are the same as the ones observed when one goes from mainland forest to Little Barrier.

From these facts we draw the following conclusions.

1) Exotic species that successfully penetrate browsed mainland climax forests with decimated native bird communities are excluded from unbrowsed island climax forests with intact native bird communities. We can speak with confidence of exclusion as opposed to nonarrival, because exotic species breed on Little Barrier immediately outside the forest.

2) We accept that mammalian predators are an important factor in the decimation of native birds, and hence in the success of exotic birds, in mainland forest. However, predators are not the whole answer: exotics penetrate the forests of Lady Alice and Mauitaha despite these islands being as predator-free as Hen and more so than Little Barrier.

3) Two observations suggest that the effect of browsing on mainland forest structure is also an important factor. First, Little Barrier's forest avifauna differs from that of the mainland not only in the absence of exotic species but also in the altered relative abundances of native species, and these alterations are mostly ones expected from lack of browsing. Second, alterations in forest structure due to succession have allowed exotic bird species to enter forest on Lady Alice, and even more on Mauitaha. The changes in relative abundances of bird species, as one proceeds from Little Barrier or Hen to Lady Alice to Mauitaha, are qualitatively similar to the changes observed along a similar successional gradient on the mainland, where exotic bird species are most dominant in the most disturbed habitats (*16*).

In short, we infer that exotic bird species were not able to achieve their present penetration into New Zealand forest until the forest structure had been disturbed by browsing and logging, or until native species had been decimated by predation, disease, and these habitat changes. (The relative importance of these two factors remains unknown.) This conclusion suggests that control of introduced mammals is crucial to the future of New Zealand's surviving avifauna in native forest.

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14. On Little Barrier, Maoris carried out clearing and selective logging in some lowland areas, now covered with regenerating *Leptospermum* forest. Our census data are from unmodified forest. The sole extant clearing is 20 ha around the resident ranger's house. Hen shows signs of Maori cultivation in the distant past but is now completely forested.
15. Absence of browsers results in more abundant, less disturbed leaf litter on the ground, denser lower and middle stories, and fewer openings, as well as in more food plants for birds. The denser forest is related to the increased abundance of *M. albicilla*, which gleans on foliage and limbs, and to the decreased abundance of *G. igata*, which prefers disturbed forest with openings. The honeyeaters. *A. melanura* and *P. novaeseelandiae* exploit native plants for fruits and nectar. Nests of *P. novaeseelandiae* and possibly *M. albicilla* are especially vulnerable to mammalian predators.
16. Does the varying penetration of exotic species into island forest depend on the varying local number of native forest bird species? This interpretation is refuted by two facts: mainland forest has about as many native bird species as Little Barrier, and Lady Alice has about as many as Hen; yet, in each case exotic species are common in forest on the first-named island of the pair but virtually absent on the second. Hence we infer that the dependence is instead on abundance of native bird species, which depends in turn on forest structure.
17. J.M.D. thanks G. R. Williams, the New Zealand Wildlife Service, and the Lievre Fund for making his studies in New Zealand possible.

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Associative Learning in *Aplysia*: Cellular Correlates Supporting a Conditioned Fear Hypothesis

Abstract. *Aversive associative learning in* Aplysia californica *survives restraint of the animal and surgical exposure of the central nervous system. The learning is expressed in the intracellularly recorded activity of identified motor neurons mediating three different defensive behaviors: escape locomotion, inking, and siphon withdrawal. In each case, animals that had previously received paired training showed significant facilitation of synaptic input to motor neurons during test stimulation in the presence of the conditioned stimulus. Animals without such training showed no facilitation of input to the motor neurons. Resting potential and input resistance appeared unaffected by conditioning and were not altered by application of the conditioned stimulus. These results show that the conditioned facilitation of defensive responses cannot be explained by subthreshold actions of the conditioned stimulus on the motor neurons and support the hypothesis that* Aplysia *learn to associate the conditioned stimulus with a fearlike central state.*

Aversive conditioning of *Aplysia californica* produces a learned response resembling conditioned fear in vertebrates. After a chemosensory conditioned stimulus (CS, shrimp extract) is paired with an aversive unconditioned stimulus (US, head shock) the CS becomes capable of enhancing defensive responses and reducing at least one appetitive response (*1*). Although one defensive behavior (head withdrawal) is directly elicited by the CS after training, three other defensive behaviors (escape locomotion, inking, and siphon withdrawal) are not elicited by the CS, yet they are significantly facilitated if they are elicited by other

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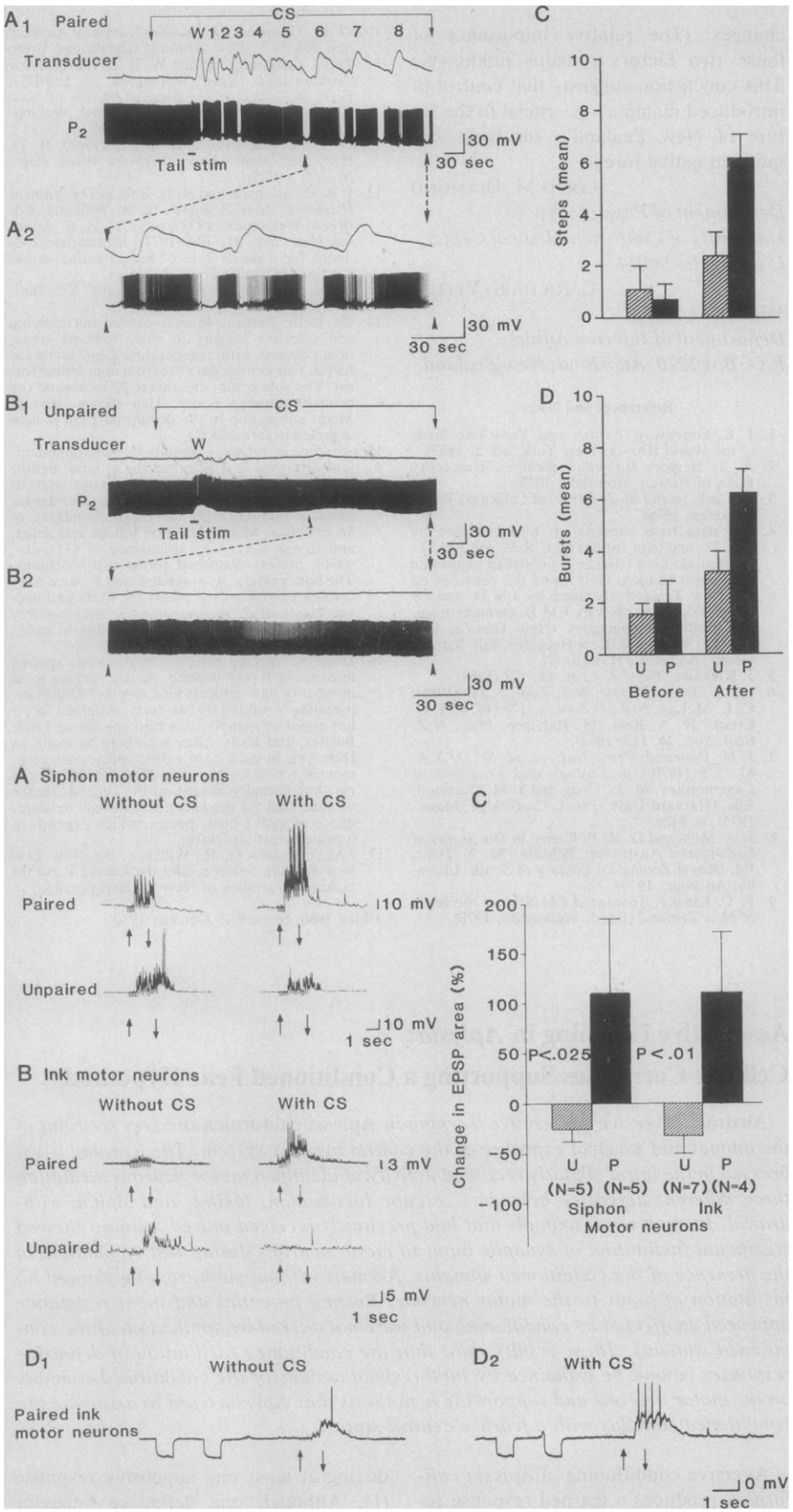

stimuli in the presence of the CS. On the basis of these results we have hypothesized that the CS elicits a conditioned fearlike state that primes a variety of defensive behaviors. However, further insight into the nature of this conditioned effect is difficult to obtain with behavioral experiments alone. For example, the facilitatory effects of the CS could be produced through either a modulation of the gain of defensive stimulus-response pathways or through direct subthreshold activation by the CS of each defensive motor system. Although these alternatives are difficult to distinguish with behavioral experiments, additional insight can be obtained by examining neurophysiological responses in identified cells of known function after conditioning. We report here that associative learning persists in restrained and surgically exposed animals and that neuronal correlates of the learning can be recorded in the identified motor neurons of each of three different defensive behaviors: escape locomotion, inking, and siphon withdrawal. Moreover, facilitation of these defensive behaviors cannot be accounted for by subthreshold effects of

Fig. 1 (top). Correlates of associative learning in pedal motor neurons. Examples of responses in a paired animal (A) and an unpaired animal (B). The CS was applied 60 seconds before the test stimulus (bar). In each part, the top trace is a record of movements of the foot recorded with a tension transducer; *W* indicates the reflexive withdrawal movement in response to the test stimulus. Successive steps are numbered. The lower trace shows the electrophysiological activity of identified motor neuron P2. (A_1 and B_1) Condensed records for the entire 5-minute period following the test stimulus. (A_2 and B_2) Part of each record on an expanded time scale to illustrate that P2 in the unpaired animal is firing tonically at a rate normally seen in quiescent unconditioned animals (*2*). By contrast, in the paired animal, P2 displays cyclic bursts correlated with locomotor movements. (C) Summary of results obtained by counting steps in the 5-minute periods before and after application of the test stimulus. Paired and unpaired animals tested in the semi-intact preparation showed significant differences in locomotor movements in the presence of the CS. (D) Summary of electrophysiological results. During the behavior shown in (C), paired animals showed significantly more bursts in pedal motor neurons than unpaired animals did. Fig. 2 (bottom). Correlates of associative learning in siphon and ink gland motor neurons. (A) Examples of synaptic responses evoked by tail stimulation (arrows) in siphon motor neurons of a paired and an unpaired animal. Relative to the response without the CS, the response with the CS was facilitated in the paired animal and depressed in the unpaired animal. Small downward deflections are shock artifacts from tail stimulation. (B) Examples of synaptic responses evoked by tail stimulation in ink gland motor neurons. As in the siphon motor neurons, the complex EPSP was facilitated in the presence of the CS in the paired animal but depressed in the unpaired animal. (C) Synaptic responses in siphon and ink gland motor neurons. Each bar represents the mean facilitation as measured by the difference in EPSP area between the test without the CS and the test with the CS present. (D) Lack of change in input resistance of ink gland motor neuron produced by application of the CS to a paired animal. Arrows indicate test stimulation following hyperpolarizing pulses. The synaptic response is facilitated by the CS, even though input resistance is unchanged.

the CS on the motor neurons. Rather, the CS exerts a common action onto all these defensive response systems at sites central to the motor neurons.

Forty-one *Aplysia californica* (100 to 400 g) were studied. All animals first received six to nine trials of either paired or specifically unpaired (control) training as previously described (*1*). Briefly, the shrimp CS was applied for 90 seconds. Sixty seconds after CS onset, the US (a 450-mA shock to the head) was applied for 30 seconds to paired animals. Unpaired animals received the same CS and US separated by 90 minutes. The intertrial interval was 3 hours. Eighteen hours after training, we examined the motor systems of escape locomotion, inking, and siphon withdrawal in a modified "split-foot" preparation (*2*, *3*), which permitted intracellular recording from identified motor neurons for each response during behavioral testing. Each animal was coded so that during dissection and experimentation the experimenter did not know the animal's training history.

To study escape locomotion, we simultaneously recorded the intracellular activity of identified motor neurons (in the pedal ganglia) that participate in escape locomotion (*2*) and the movements of the foot during each step (with a tension transducer). The CS was applied to the head (rhinophores and tentacles) for 1 minute, and then escape was triggered by delivering a weak electrical shock to the tail (*4*). The CS remained present throughout the ensuing 5-minute test period. Examples of the results from a paired and an unpaired animal are shown in Fig. 1, A and B. In the presence of the CS, paired animals ($N = 10$) showed more steps [$t(18) = 2.55$, $P < .01$] and more action potential bursts in pedal motor neurons [$t(18) = 2.12$, $P < .025$] than did unpaired animals ($N = 10$; Fig. 1, C and D). Moreover, application of the CS produced no significant alteration in the membrane potential of motor neurons in paired animals compared with unpaired animals.

It is possible, however, that a direct subthreshold effect of the CS might be expressed as an elevation of input resistance in the motor neurons of paired animals rather than as a change in membrane potential. To examine this possibility, in two additional experiments we monitored the input resistance of the motor neurons with hyperpolarizing electronic pulses injected into the soma before, during, and after presentation of the CS in paired animals. We found that significant facilitation of the locomotor program (specific to the presence of the CS) was observed with no change in input resistance of the motor neurons. Thus the facilitation of pedal motor neuron output by the CS in paired animals is not likely to be explained by direct subthreshold effects of the CS on the motor neurons (*5*).

To study inking and siphon withdrawal, we recorded intracellularly from identified ink gland and siphon motor neurons in the abdominal ganglion of 15 animals (*6*). We first applied a test stimulus (a weak electric shock) (*7*) to the tail and recorded the complex excitatory postsynaptic potential (EPSP) produced in the ink and siphon motor neurons. We then applied the CS to the head for 1 minute and delivered a second test stimulus. The amplitude and duration of the test EPSP's (*8*) in the absence and in the presence of the CS were then compared for paired and unpaired animals. Examples of the results are shown in Fig. 2, A and B. In the absence of the CS there were no significant differences between groups in the amplitude or duration of the complex EPSP's. In the presence of the CS, however, significant differences were observed (Fig. 2C). In paired animals, the test EPSP was more than doubled in both ink gland and siphon motor neurons whereas in unpaired animals, the EPSP was on average smaller in the presence of the CS. That is, compared with the unpaired animals, paired animals showed significantly greater facilitation of input to both the ink gland [$t(9) = 3.00$, $P < .01$] and siphon motor neurons [$t(8) = 2.74$, $P < .025$]. The depression of test EPSP's in the presence of the CS in unpaired animals is probably due to habituation of the second test input, although this result is also consistent with the possibility that the unpaired animals are exhibiting a form of conditioned inhibition (*9*).

As in the locomotor system, there was no significant difference in the effects of the CS on membrane potential of either the ink gland or siphon motor neurons in paired and unpaired animals. The CS occasionally produced some depolarization (average, 5 mV), especially of ink gland motor neurons, but this effect was the same in paired and unpaired animals. Moreover, as in the pedal motor neurons, significant facilitation of the test EPSP was produced by the CS in the absence of any change in input resistance or membrane potential (Fig. 2D). These results, like the previous findings on escape locomotion, show that the passive properties of the motor neurons (as reflected in the resting potential and input resistance) are unchanged by conditioning. In addition, the CS presented alone does not produce any response in the motor neurons. Rather, the CS acts to enhance synaptic input to the motor neurons produced by stimuli that trigger defensive responses.

The neuronal correlates of associative learning in *Aplysia* support and extend the conclusions derived from behavioral studies (*1*). (i) Significant correlates of the learning in three separate motor systems provide an independent confirmation on a cellular level for associative learning in *Aplysia* and indicate the utility of molluscan preparations for cellular studies of associative learning (*10*), (ii) We previously found that conditioned modulation of escape locomotion in *Aplysia* requires the presence of the CS (*1*). Consistent with this observation is our finding that neither the basic electrophysiological properties of the motor neurons nor the synaptic input are changed in paired animals compared with controls in the absence of the CS. (iii) The fact that the CS does not exert direct subthreshold effects on the motor neurons in the absence of a test stimulus supports the hypothesis that conditioning involves the learning of an association between the CS and a modulatory state rather than specific motor responses. (iv) The expression of the associative learning in identified neurons of several different motor systems promises to be useful in the search for cells that exert a common influence on each system and thus may ultimately facilitate the elucidation of the neural circuitry and cellular mechanisms underlying the conditioned central state.

A conclusive evaluation of the mechanisms by which the conditioned fearlike state is expressed will require examination of each component of these defensive stimulus-response pathways. The siphon withdrawal system holds particular promise in this regard. Preliminary experiments (*11*) indicate that a significant fraction of the complex EPSP from tail afferents may be monosynaptic to the siphon motor neurons. A monosynaptic test system has proved extremely useful in analyzing the cellular and biophysical mechanisms of nonassociative learning in *Aplysia* (*12*); the possibility now exists that the consequences of associative learning can be examined on this level as well.

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3. Animals were injected with isotonic $MgCl_2$ (50 percent of body weight), and the central nervous system was surgically exposed (*2*). In studies of siphon and ink motor neurons, the mantle organs were isolated from the body except for a connection through the pleuroabdominal connectives between the abdominal ganglion and the rest of the central nervous system. After surgery, the $MgCl_2$ was cleared by perfusing artificial seawater through the aorta for at least 1 hour.
4. Parameters of this stimulus were the same as in the behavioral studies (*1*).
5. These data do not rule out the possibility that the CS might induce changes in premotor neurons in the locomotor system of paired animals.
6. We recorded from ink motor neurons L14A or L14B [T. J. Carew and E. R. Kandel, *J. Neurophysiol.* **40**, 692 (1977)] and a novel class of siphon motor neurons that are uniquely sensitive to tail stimulation. Sometimes both classes of cells were examined simultaneously. Fifteen ganglia were examined, seven paired and eight unpaired. Siphon motor neurons were hyperpolarized to permit measurement of the complex excitatory postsynaptic potential (EPSP).
7. Weak electrical shocks (approximately 1 mA) were applied through thin wires implanted in the tail. Each train was approximately 2 seconds long, with 2-msec pulses (6 Hz).
8. The EPSP's were traced, cut out, and weighed to obtain a measure of EPSP area (a combined measure of amplitude and duration). The weights of EPSP tracings were then compared.
9. Preliminary results suggest that *Aplysia* receiving unpaired training may learn a form of conditioned inhibition—the CS becomes a "safety signal" rather than a "danger signal"—and thus the CS may inhibit defensive responses in these animals (E. T. Walters, T. J. Carew, E. R. Kandel, in preparation).
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11. Using electrical stimulation of the posterior pedal nerve (which innervates the tail) to produce a complex EPSP in the siphon and ink motor neurons, we found that 35 to 50 percent of the EPSP remained with no change in latency when the threshold of interposed interneurons was elevated by bathing the central nervous system in a solution containing high concentrations of Ca^{2+} and Mg^{2+} ions.
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Associative Learning in *Aplysia*: Evidence for Conditioned Fear in an Invertebrate

Abstract. *Aversive classical conditioning of* Aplysia californica, *a gastropod mollusk suited for neurobiological study, produces a learned reaction to the chemosensory conditioned stimulus that is expressed as a marked facilitation of four defensive responses: two graded reflexes (head and siphon withdrawal), an all-or-none fixed act (inking), and a complex fixed action pattern (escape locomotion). In addition, the conditioned stimulus produces a concomitant depression of at least one appetitive response, feeding. These extensive and selective actions of the conditioned stimulus in* Aplysia *resemble the actions of conditioned fear stimuli in higher mammals and suggest that the functional equivalent of fear occurs in invertebrates and thus may be an adaptive mechanism that is widespread in the animal kingdom.*

It has recently proven possible to analyze the cellular mechanisms of nonassociative forms of learning in several simple invertebrate preparations (*1*). The apparent generality of these mechanisms (*2*) has in turn encouraged the development of invertebrate models for the study of mechanisms of associative learning. Four independent studies have now shown that aversive associative learning occurs in gastropod mollusks, animals that offer many advantages for cellular neurobiological studies (*3*, *4*). However, an analysis of the underlying neuronal mechanisms and an assessment of the generality of these mechanisms will require an understanding of what the animals actually learn during aversive associative conditioning. In particular, one needs to know how the consequences of aversive conditioning are organized in these animals and the extent to which these effects resemble those seen in mammals after aversive conditioning.

Recently we found (*4*) that *Aplysia californica* can rapidly acquire a temporally specific aversive reaction to a chemosensory conditioned stimulus (CS) paired with a noxious unconditioned stimulus (US). This learned reaction was expressed as a facilitation of escape locomotion triggered in the presence of the CS. Because the CS did not elicit obvious conditioned responses after training, we proposed that the animals might have learned to associate the CS not with specific motor responses but instead with a central defensive state that modulates escape locomotion. To test this interpretation, we have examined the effect of the CS on four defensive responses: two graded reflex acts (head and siphon withdrawal), an all-or-none fixed act (inking), and a complex fixed action pattern (escape locomotion). In addition we have examined the effects of the CS on an appetitive behavior, feeding. We have found that aversive conditioning in *Aplysia* produces a conditioned internal state which appears functionally equivalent to conditioned fear in mammals.

One hundred and seventeen *A. californica* were studied. Animals were trained according to procedures previously described (*4*). Paired animals received the CS (shrimp extract for 90 seconds) 1 minute before the onset of the US (electric shock to the head for 30 seconds) (*5*). Unpaired controls received the same CS 90 minutes after the US. In the experiments shown in Fig. 1, paired ($N = 17$) and unpaired ($N = 16$) animals received nine trials, three trials per day, with an intertrial interval of 3 hours. During training, all animals responded to the US with maximal withdrawal of the head; secretion of ink, opaline, and mucus; siphon withdrawal; turning away from the shock, and (after a delay of several minutes) escape locomotion. Animals were then tested 1 and 2 days after the last day of training (*6*). In each test session, the CS was delivered to the head and left in the chamber for the entire test period (6 minutes). In all tests, the observer was unaware of the identities of the animals.

We first examined head withdrawal, the only overt response elicited by the CS after conditioning. We had not previously seen significant differences between paired and unpaired animals in the incidence of CS-evoked head withdrawals after conditioning (*4*), and we had observed that the CS produced weak withdrawals in untrained animals. To see if conditioning might produce a difference in the intensity of head withdrawal to the CS, we rated the magnitude of each withdrawal on a three-point scale: strong, weak, or no withdrawal. Significantly more paired animals withdrew strongly from the CS than unpaired animals did (Fig. 1A) (Fisher exact probability test, $P < .01$). Similar differences were noted in separate experiments (*7*) ($N = 12$ per group) in which the amplitude of head withdrawal [in a restrained preparation (*8*)] was measured with a tension transducer. Thus, although an unconditioned head withdrawal response to the CS can be seen in both untrained and unpaired animals, aversive conditioning significantly facilitates the amplitude of this withdrawal in animals receiving paired training.

Other defensive responses do not seem to be directly elicited by the CS after training, but the effects of conditioning can be seen on these responses when test stimuli are used to trigger them

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