Habituation: Regulation through Presynaptic Inhibition

Abstract. *During tail-flip escape responses of crayfish, synaptic transmission at the habituation-prone synapses of the lateral giant reflex pathway is presynaptically inhibited. This prevents transmitter release and all subsequent postsynaptic actions and spares the reflex from becoming habituated to stimuli produced by an animal's own escape movements. These observations demonstrate the existence of a control circuit whose adaptive function is to regulate the malleability of inherently plastic synapses. They also suggest that regulation of plasticity could be a common use of presynaptic inhibition.*

The neuronal circuit which mediates "tail-flip" escape responses to sudden tactile stimulation of the abdomen in crayfish has been elucidated in terms of identified neurons (Fig. 1) (*1–3*). This escape reflex habituates readily, and the habituation is due to either decreased transmitter output or synapse-specific postsynaptic desensitization (hereafter called collectively "synaptic depression") at the first, chemical, synapses of the reflex arc (Fig. 1) (*1, 4*).

The ability of this reflex to habituate is presumably of adaptive value to the crayfish. However, it presents the animal with a special problem. Whenever animals swim via rapid abdominal flexions—which they do, not only during escape from abdominal contact but also under other circumstances (*5*)—many of the sensitive tactile afferents of the reflex will fire. Since the synapses between these afferents and the first-order interneurons of the reflex are labile, they would be expected to become depressed during tail-flip locomotion and to leave the animal unable to react to subsequent threats. This problem is acute, because even stimuli occurring as infrequently as once in 5 minutes cause habituation, which then can persist for many hours (*4*); furthermore, swimming sometimes involves long sequences of repetitive tail flips, which would be expected to depress the first-order synapses particularly severely. There thus arises the need for a mechanism which can protect these synapses from change during tail flips.

We report here that protection from synaptic depression does occur and is due to a pathway that appears to operate by presynaptically inhibiting the afferent terminals of depressable synapses during tail-flip responses (*6*).

The phenomena discussed here are mediated by the abdominal nerve cord (*1–5*). In our experiments this region was isolated from the remainder of the nervous system and was either disconnected from flexor musculature or entirely excised to prevent movement. Stimuli were single shocks to ganglionic roots containing sensory fibers. The degree of escape reflex excitation was measured by the size of intracellularly recorded excitatory postsynaptic potentials (EPSP's) evoked in the cord's lateral giant (LG) fibers (Fig. 1), which are the command neurons for the reflex (*7*). In order to determine the extent to which the reflex was protected from depression due to tail-flip responses, we activated the motor circuitry for such responses by directly stimulating either the medial giant fibers of the nerve cord (which normally drive tail flips in response to visual or rostral tactile stimulation) or the LG's themselves (*8*).

When stimuli which initially evoke a large EPSP in the LG are repeated at 5-second intervals, there is a 25 to 35 percent diminution of EPSP amplitude within a few trials (trials 1 to 11, Fig. 2A) due to synaptic depression of the reflex's first tier of chemical synapses. This effect is the physiological correlate of behavioral habituation of the reflex. However, if an identical sequence of stimuli is presented, but each stimulus is preceded by activation of tail-flip motor circuits, little or no depression occurs (trial 11*, Fig. 2A).

We have also carried out behavioral experiments on animals implanted with permanent giant fiber stimulation and recording electrodes in order to verify that neither tactile stimuli delivered during behavioral tail-flip responses nor repeated tail flips themselves (evoked by direct giant fiber stimulation) produce habituation in intact animals; identical tactile stimulation given alone causes marked habituation, which lasts for more than 2 hours.

These effects cannot be due to sensitization of reflex excitability or "dishabituation" of the reflex, such as has been described in other systems (*9*), because (i) repeated activation of tail-flip motor circuitry by itself does not cause an increase of escape reflex excitability, (ii) repeated activation of tail-flip motor circuitry in an already depressed preparation in no way facilitates recovery from depression, and (iii) stimuli must occur specifically at the time when an intact animal would be making a power stroke (10 to 40 msec after activation of tail-flip circuitry) for the effect to be maximal. We therefore consider that the first tier of synapses has been protected from synaptic depression.

It seemed plausible that such protection might be accomplished by presynaptic inhibition of transmitter release from the afferent terminals of the labile synapses, thus minimizing highly probable causes of synaptic depression (*1, 4*) such as transmitter depletion and postsynaptic desensitization of those synapses. It has for some time been known that the escape reflex is in fact inhibited after activation of tail-flip motor circuitry (*10*), and the magnitude of protection at various times after motor circuit activation does correlate almost perfectly with the magnitude of this inhibition (Fig. 2B). However, heretofore the inhibition of the reflex has been attributed exclusively to postsynaptic inhibition of the LG command neuron itself (pathway 3, Fig. 1) where it should not be able to influence synaptic depression at the first tier of synapses. Therefore, evidence of presynaptic inhibition of transmission across the first tier of synapses was sought by recording directly from first-order interneurons of the escape circuit. Consistent with the hypothesis of primary afferent inhibition, extracellular recording from several first-order tactile interneurons showed that both inhibition of transmission and protection from depression occur at the initial synapses of the reflex arc.

In order to determine more directly whether presynaptic inhibition was operating, intracellular analysis was carried out on the largest of the identified first-order interneurons (Fig. 1, interneuron A), which was impaled near the initial segment of its main axon (*11*). In this neuron a sequence of ten stimuli delivered 5 seconds apart normally resulted in a 10 to 30 percent reduction in amplitude of evoked EPSP's. However, when activation of tail-flip motor circuits preceded each stimulus by 20 to 30 msec, there was virtually no habituation. Accompanying this protection was a profound inhibition of transmission (Fig. 2, C_1 and C_3) whose magnitude correlated closely with that of the protection (Fig. 2D).

Whereas the correlated occurrence of protection and inhibition in this first-order interneuron suggests the operation of presynaptic inhibition, the intracellular records show an obvious postsynaptic input following activation of tail-

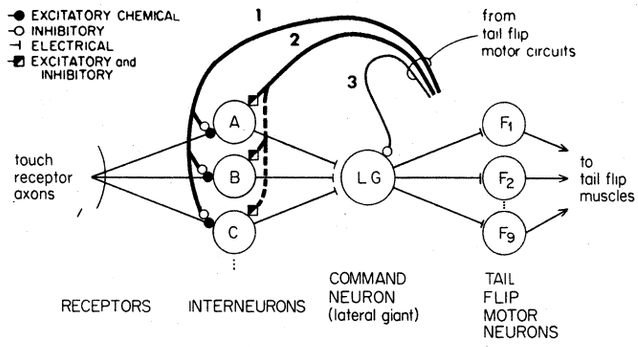

Fig. 1. Crayfish lateral giant escape reflex circuit (*1–3*). The basic circuit, in terms of named neurons, is indicated at the bottom of the figure. Feedback pathways (specific neurons not yet identified) activated by tail-flip motor circuits are indicated by pathways 1 to 3 at the top of the figure. Pathway 1 is the focus of this report. Thin lines indicate formerly established circuitry; bold lines, circuitry reported here; dashed lines, circuitry conjectured on the basis of symmetry. Note that motor neurons F_1, and so on, can be driven by other command systems not shown. Also, cross connections between interneurons A, B, and so on, monosynaptic connections to LG, and the inhibitory motor neuron F_{10}, which are not relevant to this report, are omitted.

flip motor circuits (Fig. 2, C_2 and C_3; pathway 2, Fig. 1). The depolarizing phase of this biphasic effect can cause firing when it is added to a subthreshold EPSP, and so is itself probably an EPSP. The subsequent hyperpolarization is an inhibitory postsynaptic potential (IPSP), which is associated with a measured conductance increase of 30 percent. This postsynaptic inhibition is potent enough to rapidly repolarize an EPSP during its rising phase (Fig. 2, C_4 and C_5), and might have been thought sufficient to account for the inhibition of transmission at this synapse.

However, it seemed extremely unlikely to us that postsynaptic events could be responsible for protection, and a careful comparison of the time courses of the IPSP, functional inhibition, and protection supports this view. In each of six experiments both inhibition and protection outlasted the IPSP by 20 to more than 100 msec, and on the average both were still at 30 percent of their maximum levels as the IPSP returned to baseline (Fig. 2D). Thus, both inhibition and protection can occur in the absence of any visible postsynaptic effect. This result supports the notion that presynaptic inhibition (pathway 1, Fig. 1) is operating and is responsible for protection.

If this conclusion is correct, one would expect conductance increases and perhaps depolarizations in tactile sensory axon terminations during inhibition (*12, 13*), and these might alter the extent to which focal shocks near these terminations could evoke antidromic spikes in the axons of origin. In fact antidromic volleys set up in a sensory root which feeds interneuron A were changed in size (Fig. 2E) when evoked after activation of tail-flip motor circuits (*14, 15*). Most often decreases (as in Fig. 2E) were found, which suggests that the result is attributable to the shunting effects of terminal axon conductance increases rather than to terminal depolarization (*16*). The time course of these effects paralleled that of total inhibition and of protection rather than that of the IPSP in interneuron A.

In this instance, as in others, it has not been possible to obtain absolute

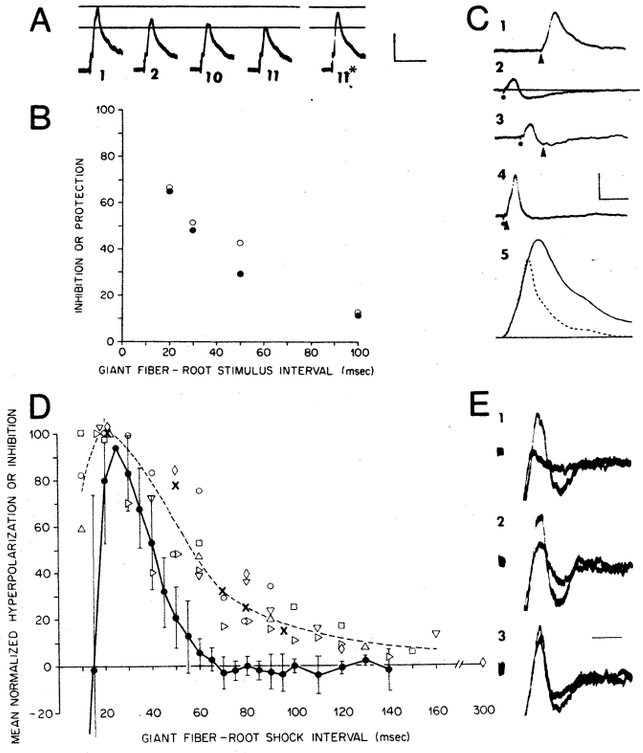

Fig. 2. (A) Excitory postsynaptic potentials (EPSP's) in LG during repeated stimulation. Eleven trials at one per 5 seconds produce rapid depression (trials 1, 2, 10, and 11 are shown). These provide a control for the next manipulation. After recovery, 11 stimuli at 1 per 5 seconds were again presented, but each of the first 10 stimuli was preceded by activation of tail-flip motor circuitry (via intracellular medial giant shocks). There is a relative lack of depression on trial 11* as compared to trial 11. Stimulus electrodes were on the second root of the third abdominal ganglion (calibration, 4 mv and 20 msec). (B) Time course of inhibition and protection. Percent inhibition is defined as 100 (1 — inhibited EPSP/control EPSP), where EPSP magnitude is measured from any IPSP present in the LG as a baseline (see text). Percent protection is defined [referring to format of (A)] as, 100 (trial 11* EPSP — trial 11 EPSP)/(trial 1 EPSP — trial 11 EPSP). Open circles indicate mean percentage inhibition; closed circles indicate mean protection. (C) Postsynaptic potentials in interneuron A. C_1, EPSP evoked by shocking afferent fibers (first root, last ganglion). C_2, Response to activation of tail-flip motor circuitry. C_3, Inhibition of EPSP: the motor circuitry was activated at the dot; stimulus of C_1 at triangle. C_4, Repolarization due to IPSP; the symbols are as in C_3; the IPSP is timed to start during the probable time of maximum excitatory transmitter action. C_5, Graphical superimposition of C_4 minus C_3 (dashed) on C_1 (calibration, 2 mv, 20 msec). (D) Time course of hyperpolarization (closed circles), total inhibition (open symbols), and protection (crosses) in interneuron A. Afferent stimuli delivered via first root of last ganglion. For hyperpolarization measurements the abscissa values represent the times since giant fiber firing. Percentage inhibition and protection were calculated as in (B). All measures were normalized to 100 at their maximum value in each experiment. Hyperpolarization and protection are given as means; total inhibition is given for individual preparations (different symbols). Error markers are standard deviations over six preparations. (E) Antidromic sensory root responses during inhibition. Compound action potentials in the fourth (sensory) root of the last ganglion were elicited by shocking the terminal region of the root via a 5-μm stainless steel electrode in neuropile. Antidromic stimuli were given 20 msec (E_1), 80 msec (E_2), and 100 msec (E_3) after tail-flip motor circuit activation. In each case control responses to antidromic stimulation alone (the larger response) is superimposed on the test response (calibration, 1.0 msec).

proof of the existence of presynaptic as opposed to remote postsynaptic inhibition. However, we think that, when all the facts reported above are considered together, it becomes very difficult to escape the conclusion that presynaptic inhibition is operative here and that it functions to protect the first synapse of the reflex pathway from depression during tail-flip responses, thereby protecting the animal from maladaptive habituation.

The theoretical possibility of a protective mechanism based on presynaptic inhibition would be predicted from most currently favored explanations of presynaptic inhibition and synaptic depression (*17*). Similarly, protection from postsynaptic potentiation and temporal facilitation seem theoretically possible, and the latter has been found (*18*).

We believe the importance of these observations to be twofold. (i) Intrinsic changes of synaptic efficacy which play an important role in normal functioning are not obligatory. There are mechanisms which can operate to prevent them specifically when they would be maladaptive. (ii) The role of presynaptic inhibition in achieving such regulation of inherent plasticity suggests a new and perhaps rather general function for presynaptic inhibition in the nervous system.

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References and Notes

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6. The methods used permit this conclusion only for tail flips mediated by medial or LG fibers. The tail flips which occur during swims composed of long sequences of flips are mediated by a nongiant system which has not been studied here. However, J. J. Wine and F. B. Krasne (in preparation) have found that inhibition of both LG and interneuron A can be activated by tail-flip motor neurons; we therefore consider it extremely likely that protection accompanies all tail-flip responses.
7. Stimulation and gross recording were carried out by hook or suction electrodes. Micropipettes were filled with 2.5*M* KCl. The LG's were impaled just rostral to septa in third or fourth abdominal ganglia; EPSP's were evoked by second-root shocks. Preparations were irrigated with a constant flow of cold, aerated Ringer solution and stimuli were kept well spaced whenever possible to minimize deterioration [for further details of methods, see (*1–4*)].
8. Shocks just suprathreshold for firing any giant fiber, delivered by pairs of platinum wires on the cord's dorsal surface, were used routinely. However, we used selective stimulation to verify that each giant fiber could by itself evoke protection from LG habituation and the early excitation and subsequent inhibition of interneuron A.
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11. In extracellular experiments, interneuron A was identified by (i) its receptive field (*1, 2*), (ii) the large size of its action potential at the ventral surface of desheathed connectives, (iii) its low threshold for activation by electrical shocks to any of ipsilateral roots 1 to 4 of the last abdominal ganglion, and in some experiments by (iv) its large diameter and (v) position in the cord. In intracellular experiments criteria (ii), (iii), and (v) were used. Impalement was at the point where the axon enters the last ganglion.
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14. The fourth root of the last abdominal ganglion, which was used for these experiments, contains a single efferent fiber and otherwise appears to be entirely sensory (*15*). Neuropile about halfway laterally from the midline at the level of entrance of the first root—where the terminal tuft of the fourth-root fibers overlaps the dendritic arbor of interneuron A [(*15*); A. Selverston and D. Kennedy, *Endeavour* **28**, 107 (1969)]—was explored with 5- to 30-μm steel electrodes for points where antidromic potentials could be evoked that were (i) gradable with stimulus current (which was then set for an intermediate-sized response) and (ii) subject to change of size when evoked after the firing of a giant fiber. Such points were not difficult to find. These changes were gradable and therefore could not be solely due to the single, fourth-root efferent.
15. Cobalt dye studies of fourth root by Ron Calabrese (personal communication).
16. Work on presynaptic inhibition at neuromuscular synapses in crayfish has indicated that the effects of decreased membrane resistance are of major importance, whereas terminal depolarization is probably slight (*12*). Decreased excitability of sensory axons during presynaptic inhibition has been observed at eighth-nerve Mauthner cell synapses in goldfish [T. Furukawa, Y. Fukami, Y. Asada, *J. Neurophysiol.* **26**, 759 (1963)].
17. This follows if these induced changes of efficacy are due to events which depend upon Na^+-spike-elicited Ca^{2+} influx into terminals [W. J. Betz, *J. Physiol.* **206**, 629 (1970); R. Miledi and R. Thies, *ibid.* **212**, 245 (1971)], since presynaptic inhibition should reduce such influx (*12, 13*). If changes of efficacy are a consequence of events that do not depend on Ca^{2+} influx [R. G. Sherman and H. L. Atwood, *Science* **171**, 1248 (1971); J. Bruner and D. Kennedy, *ibid.* **169**, 92 (1970)], predictions are equivocal.
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19. Supported by NINDS grant NS-08108. J.S.B. is a PHS trainee. We thank Drs. J. J. Wine, J. S. Liebeskind, D. Novin, G. Ellison, and A. Stuart for suggestions. We thank L. Chen for help in behavioral experiments.

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Rapid Viral Induction of Plasmacytomas in Pristane-Primed BALB/c Mice

Abstract. *Strain BALB/c mice were injected intraperitoneally with 0.5 milliliter of pristane, and 39 to 56 days later they were infected with Abelson murine leukemia virus, which is a lymphosarcomagenic variant of Moloney virus. Fifty-eight percent of the mice developed lymphosarcoma, and 28 percent developed immunoglobulin-producing plasmacytomas within 20 to 93 days (77 to 149 days after the pristane injection). Two of 57 control mice developed plasmacytomas at days 138 and 166 after a single injection of pristane; no plasmacytomas were found in mice treated with virus alone.*

The immunoglobulin-producing, transplantable murine plasmacytomas have proved valuable in elucidating the structure and function of antibodies and the mechanisms by which they are synthesized (*1*). Although spontaneous plasmacytomas are rare, they can be induced in BALB/c mice by the intraperitoneal implantation of various materials, including solid plastics, mineral oils, and chemically pure oils such as 2,6,10,14-tetramethylpentadecane (pristane). These agents induce a diffuse peritoneal granuloma in which plasmacytomas develop after 150 to more than 600 days (*1–3*). Endogenous viruses have been suspected of playing a role in plasmacytoma development. However, aside from the presence of C-type and intracisternal A-type particles (*4*), little

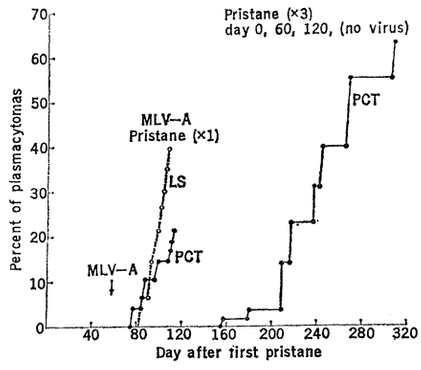

Fig. 1. Cumulative percentage of mice given a single intraperitoneal injection of pristane on day 0 and infected with Abelson virus (*MLV-A*) on day 57 that developed lymphosarcomas (*LS*) or plasmacytomas (*PCT*) in experiment 2. For comparison the cumulative percentage of mice in a previous experiment that developed plasmacytomas following three intraperitoneal injections of pristane given on days 0, 60, and 120 is shown.