through small yellow sectors (Fig. 1G). Since a single layer of green (*Rp1*) cells may be associated with some very narrow sectors or the boundaries of larger sectors (*7*), we cannot definitively determine whether the necrotic regions in these yellow sectors derive from oil yellow (*rp1*) cells. The hypersensitive necrosis initiated in green tissue at a green-yellow boundary progressed less far into oil yellow sectors than into green tissues (Fig. 1, F and G), leading to an asymmetric HR lesion. This suggests input of the *Rp* locus into propagation as well as initiation of the necrotic response. We do not believe that this result is due to reduced vigor in the yellow sectors, since the size and shape of hypersensitive lesions in *Rp1 oy/rp1 oy* and *Rp1 Oy/rp1 oy* tissues were similar (compare Fig. 1A with Fig. 1I). In this regard, however, the oil yellow sectors in the irradiated material were lighter and more homogeneously yellow than homozygous recessive oil yellow tissues. This indicates that either the hemizygous (*−/oy*) phenotype is more severe than the homozygous recessive oil yellow state or that there is a significant contribution by the hemizygosity of other loci on the short arm of chromosome 10 to the lowered vigor and yellowing of these sectors.

Our data indicate that the *Rp1* locus must be present and active in cells encountered by *P. sorghi* to determine resistance to this pathogen and, hence, that the *Rp1*-specified resistance events are not initiated by a diffusible factor. In experiments that juxtapose resistant and susceptible tissues, various groups have observed hypersensitive necrosis at the graft junction (*9*). Among the difficulties in interpreting these results are the resistance-necrosis reactions induced by cutting and gluing the grafted tissues, the lack of sharpness in (and, often, difficulty in identifying) the boundaries of resistant and susceptible tissues, and the other genetic differences between host and graft tissue sources.

The well-marked, isogenic nature of the sectored plants used in this study proves that a factor on the short arm of chromosome 10, presumably *Rp1*, specifies cell-autonomous initiation of HR-associated resistance upon exposure to *P. sorghi* spores. Determining whether or not *Rp1*-initiated hypersensitive necrosis may be propagated through cells lacking *Rp1* will require detailed microscopic analysis of fungal growth and cell necrosis at green-yellow boundaries.

REFERENCES AND NOTES

1. K. W. Shepherd and G. M. E. Mayo, *Science* **175**, 375 (1972); A. H. Ellingboe, in *Encyclopedia of Plant Pathology*, R. Heitefuss and P. H. Williams, Eds. (Springer-Verlag, Berlin, 1976), p. 761; W. Q. Loegering, *Annu. Rev. Phytopathol.* **16**, 304 (1978).
2. K. O. Müller, in *Plant Pathology*, J. G. Horsfall and A. E. Dimond, Eds. (Academic Press, New York, 1959), p. 469.
3. H. H. Flor, *Annu. Rev. Phytopathol.* **9**, 275 (1971).
4. V. H. Rhoades, *Proc. Natl. Acad. Sci. U.S.A.* **21**, 243 (1935); D. R. Wilkinson and A. L. Hooker, *Phytopathology* **58**, 605 (1968).
5. W. H. Eyster, *Am. Nat.* **67**, 75 (1933).
6. Y. Hotta and S. Benzer, *Nature* **240**, 527 (1972).
7. D. M. Steffensen, *Am. J. Bot.* **55**, 354 (1968); E. H. Coe, Jr., and M. G. Neuffer, in *The Clonal Basis of Development*, S. Subtelny and I. M. Sussex, Eds. (Academic Press, New York, 1978), p. 113; R. S. Poethig, in *Contemporary Problems in Plant Anatomy*, R. A. White and W. C. Dickison, Eds. (Academic Press, New York, 1984), p. 235; R. S. Poethig, E. H. Coe, Jr., M. M. Johri, *Dev. Biol.* **117**, 392 (1986).
8. J. L. Bennetzen, M.-M. Qin, S. Ingels, A. H. Ellingboe, *Nature* **332**, 369 (1988).
9. D. R. Jones and B. J. Deverall, *Physiol. Plant Pathol.* **12**, 311 (1978); S. R. Mircetich and J. W. Hoy, *Phytopathology* **71**, 30 (1981); S. R. Mircetich and A. Rowhani, *ibid.* **74**, 423 (1984).

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Behavioral Dissociation of Dishabituation, Sensitization, and Inhibition in *Aplysia*

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Three forms of nonassociative learning (habituation, dishabituation, and sensitization) have commonly been explained by a dual-process view in which a single decrementing process produces habituation and a single facilitatory process produces both dishabituation and sensitization. A key prediction of this view is that dishabituation and sensitization should always occur together. However, we show that dishabituation and sensitization, as well as an additional process, inhibition, can be behaviorally dissociated in *Aplysia* by (i) their differential time of onset, (ii) their differential sensitivity to stimulus intensity, and (iii) their differential emergence during development. A simple dual-process view cannot explain these results; rather, a multiprocess view appears necessary to account for nonassociative learning in *Aplysia*.

THE RELATION AMONG DIFFERENT forms of learning has been the subject of considerable debate for several decades (*1*). For example, until recently (*2*, *3*) it has been thought that three different forms of nonassociative learning (habituation, dishabituation, and sensitization) could be explained by the interactions of two opponent processes—a single decrementing process that gives rise to habituation and a single facilitatory process that gives rise to both dishabituation (the facilitation of decremented responses) and sensitization (the facilitation of nondecremented responses) (*4*). If this view is correct, then dishabituation and sensitization should always occur together. However, we report here that dishabituation and sensitization, as well as an additional process, inhibition, can be behaviorally dissociated in the siphon withdrawal reflex of the marine mollusk *Aplysia*. This reflex has been used successfully to analyze both nonassociative and associative learning on behavioral and cellular levels (*5*). Our results suggest that a simple dual-process view involving a single decrementing and a single facilitatory process requires revision and that a multiprocess view, perhaps involving inhibitory as well as facilitatory interactions, is necessary to account for the mechanisms underlying nonassociative learning.

To examine dishabituation, we first produced habituation of the siphon withdrawal reflex by administering 20 water-jet stimuli to the siphon at a 30-s interstimulus interval (ISI). We then administered a single stimulus to the tail; this stimulus ranged in intensity from a mild tactile stimulus to multiple electrical shocks (*6*). Finally, we tested the reflex amplitude with water-jet stimuli to assess the magnitude of dishabituation (*7*).

To analyze the time of onset of dishabituation, we compared two conditions. In one condition (Dishab.) animals received tail stimulation after habituation of the siphon withdrawal reflex, while in the other (Recovery) there was no tail stimulus after habituation (*8*). Groups were tested 90 s, 10 min, and 20 min later. The results for Weak

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tail shock are shown in Fig. 1A (*9*). In the 90-s test, the Recovery group showed no significant increase in response magnitude compared to the last response in the habituation series; in contrast, there was a significant increase in the Dishab. group ($P < 0.005$). Moreover, the Dishab. group showed significantly increased responses compared to the Recovery group ($P < 0.02$). Thus, significant dishabituation appeared soon (within 90 s) after the presentation of the tail stimulus. There were also significant increases in response magnitude in the Dishab. group in the later tests, but these were paralleled by comparable increases in the Recovery group (Fig. 1A). Furthermore, the increased responses of the Dishab. and Recovery groups in the 10- and 20-min tests were no longer significantly different from each other, suggesting that the increases observed at these times were due to recovery and not dishabituation. Therefore, all subsequent analyses of dishabituation were restricted to the 90-s test, where we could be confident that any increased responses after the tail stimulus were due solely to dishabituation.

Having established that dishabituation has an early onset, we then examined how dishabituation varied as a function of the intensity of the tail stimulus. Five groups of animals were compared in which the intensity of the tail stimulus was varied from a mild tactile stimulus (Touch), to increasing amplitudes of a single electrical shock [Weak, Intermediate (Int.), and Strong], and finally to multiple tail shocks (4×) (*6*). We found that the intensity of the tail stimulus had a dramatic effect on the magnitude of dishabituation observed (Fig. 1B). Significant dishabituation was produced by Touch ($P < 0.001$), Weak ($P < 0.005$), and Int. ($P < 0.001$) tail stimuli; but no significant dishabituation was produced by either the Strong or 4× stimuli. Thus, surprisingly, the magnitude-intensity relation for dishabituation showed an inverted U–shaped function, with the greatest amount of dishabituation produced by the weaker stimuli and progressively less dishabituation produced as the intensity of the tail stimulus was increased.

The second series of experiments examined sensitization. In parallel to the studies examining dishabituation, we determined both the time of onset of sensitization and its magnitude as a function of tail stimulus intensity. The procedure for analyzing sensitization was the same as that for dishabituation, except that animals were presented with only two baseline siphon stimuli at a nondecrementing (10 min) ISI prior to the tail stimulus, and an additional siphon test was added 30 min after the tail stimulus. To determine the time of onset for sensitization, we used the same range of stimulus intensities as before (*6*) and examined reflex responsiveness at a number of test intervals after the tail stimulus. In contrast to dishabituation, sensitization was not expressed until a considerable delay after the tail stimulus (Fig. 2). At both the 90-s and 10-min tests, there was no significant increase in reflex responsiveness for any stimulus intensity; in fact, there was a tendency for nondecremented reflex responses to be inhibited after the tail stimulus. Significant sensitization was only observed 20 and 30 min after the tail stimulus, indicating that sensitization has a delayed onset (*10*).

We next examined the relation between the magnitude of sensitization and tail stimulus intensity. We found that significant sensitization was only produced by stronger stimuli (Int., $P < 0.05$; Strong, $P < 0.02$; and 4×, $P < 0.01$) (Fig. 2). No sensitization was produced by weaker stimuli [Touch and Weak, not significant (NS)]. This finding is in marked contrast to that obtained for dishabituation in which significant facilitation was seen only with weaker stimuli and no facilitation was seen with stronger stimuli (Fig. 1B).

Our results show that dishabituation and sensitization can be dissociated by their time of onset and their stimulus requirements in adult *Aplysia*. Recently, in juvenile *Aplysia*

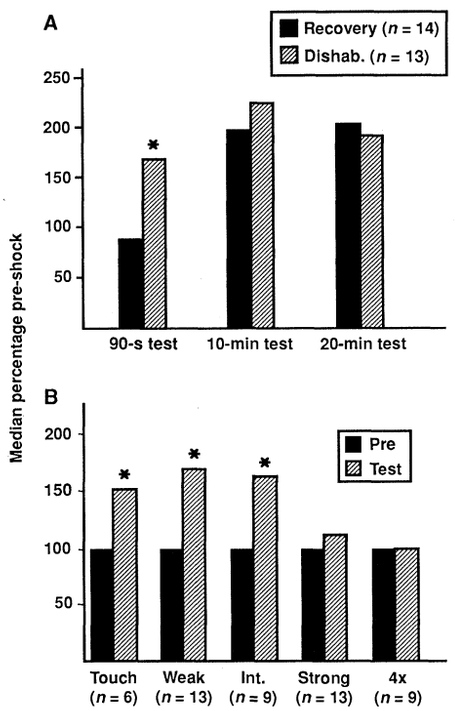

Fig. 1. Dishabituation has an early onset and is preferentially produced by weak stimuli. (**A**) Time of onset. Data from the Weak stimulus group are illustrated [comparable results were obtained for the other stimulus intensities (*9*)]. Data are normalized to the last response in the habituation series. In this and subsequent figures data are given as medians (interquartile ranges are given below in parentheses), and asterisks indicate statistical significance [as described in (*7*) and in the text]. Test results were as follows: 90-s test: Dishab. group, 169% (97%, 224%) and Recovery group, 89% (74%, 123%); 10-min test: Dishab. group, 226% (133%, 297%) and Recovery group, 196% (121%, 300%); 20-min test: Dishab. group, 193% (152%, 271%) and Recovery group, 204% (113%, 261%). (**B**) Sensitivity to stimulus intensity. Tail stimulus intensities are shown in increasing order. Data are the median responses (normalized to the habituated response) for each intensity at the 90-s test. Touch: 152% (127%, 173%); Weak: 169% (97%, 224%); Int.: 163% (114%, 191%); Strong: 111% (66%, 296%); and 4×: 100% (68%, 115%).

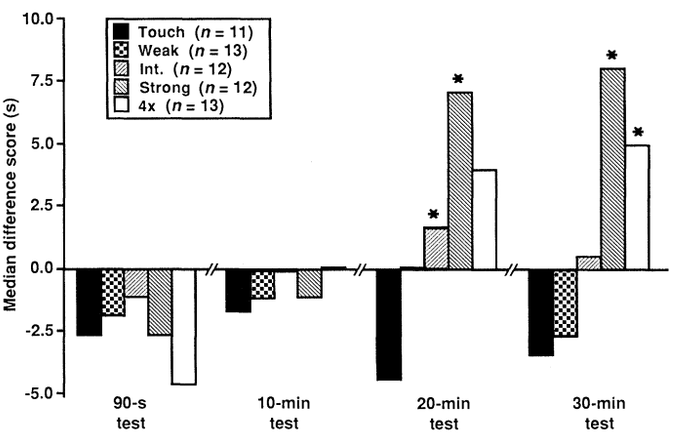

Fig. 2. Sensitization has a delayed onset and is preferentially produced by strong stimuli. The data for each of the four post-shock tests in the sensitization procedure are shown. For each test, histograms represent data from increasing stimulus intensities. The data are expressed as median difference scores in seconds (post-test minus the mean of the two pretests). At both the 90-s and 10-min tests, there is no sensitization [90-s test: Touch, −2.7 (−8.1, 0.3); Weak, −1.9 (−7.9, 1.2); Int., −1.1 (−5.5, 3.2); Strong, −2.6 (−7.3, 0.1); and 4×, −4.6 (−7.3, −3.4). Ten-minute test: Touch, −1.7 (−5.8, 2.2); Weak, −1.2 (−4.4, 2.4); Int., 0 (−2.5, 6.1); Strong, −1.2 (−4.7, 5.3); and 4×, 0.1 (−5.91, 2.71)]. Significant sensitization is only observed in the 20- and 30-min tests in response to stronger shock intensities [20-min test: Touch, −4.4 (−9.3, 4.3); Weak, 0.1 (−4.9, 4.5); Int., 1.7 (0, 5.5); Strong, 7.1 (0.4, 19.2); and 4×, 4 (−2.2, 6.8)]. Thirty-minute test: Touch, −3.4 (−9.4, −0.4); Weak, −2.7 (−9.6, −1.2); Int., 0.5 (−3.6, 5.6); Strong, 8 (−1.3, 17.4); and 4×, 5 (2.1, 12.6)].

we have also found that dishabituation and sensitization can be dissociated on the basis of their developmental timetables (*11–13*). These three independent means of distinguishing between dishabituation and sensitization are summarized in Fig. 3. The dissociation of dishabituation and sensitization on the basis of their differential time of onset is most apparent shortly after the tail stimulus. We compared the effects of a range of tail stimulus intensities on habituated and nonhabituated responses in the 90-s test (Fig. 3A). Dishabituation is exhibited at a number of stimulus intensities, whereas in this early test sensitization is not produced at any intensity. In fact, the strongest tail stimulation produces significant inhibition of nondecremented responses (4×, $P < 0.01$). This same stimulus intensity (4×) produced no dishabituation. Thus the lack of dishabituation to the 4× stimulus may be due to the recruitment of an inhibitory process that competes with the expression of the facilitatory process of dishabituation (*14*). Dishabituation and sensitization can also be dissociated by their sensitivity to stimulus intensity (Fig. 3B). Maximal dishabituation is produced by weak stimuli; maximal sensitization is produced by strong stimuli. Moreover, the stimulus that is most effective in producing dishabituation (Weak) produces no significant sensitization, and the stimulus that is most effective in producing sensitization (Strong) produces no significant dishabituation. Finally, dishabituation and sensitization emerge at different times during development (Fig. 3C). Previous work had shown that dishabituation emerges early in development (about 2 weeks after metamorphosis) (*11–13*, *15*), but that sensitization is not apparent until about 50 days later (*11–13*). Thus, the two forms of learning can be distinguished on the basis of their ontogenetic timetables.

In summary, we have determined that dishabituation and sensitization in *Aplysia* can be dissociated in three independent ways: on the basis of their time of onset, their stimulus requirements, and their developmental timetables. These behavioral observations raise important questions about the cellular processes underlying the dissociation of these two forms of learning. One possibility is that dishabituation and sensitization may reflect different underlying mechanisms. For example, the temporal dissociation between the onset of dishabituation and sensitization may reflect the differential activation of two processes, one which turns on rapidly and produces facilitation of decremented responses, and another which turns on gradually and produces facilitation of nondecremented responses. An alternative possibility is that the behavioral dissociation we observe is produced by the inhibitory process initiated by tail shock. For example, nondecremented processes may be more susceptible to inhibition, and thus the delayed onset of sensitization may reflect the gradual wearing off of the inhibitory process that competes with or masks the expression of sensitization. From our behavioral results, one cannot distinguish between these possibilities. However, recent progress has been made in elucidating the cellular mechanisms underlying dishabituation and sensitization (*3*, *16*) as well as inhibition (*17*, *18*). Thus, it will now be important to determine the degree to which these different mechanistic processes can account for the behavioral dissociation that we have observed in both developing and adult animals.

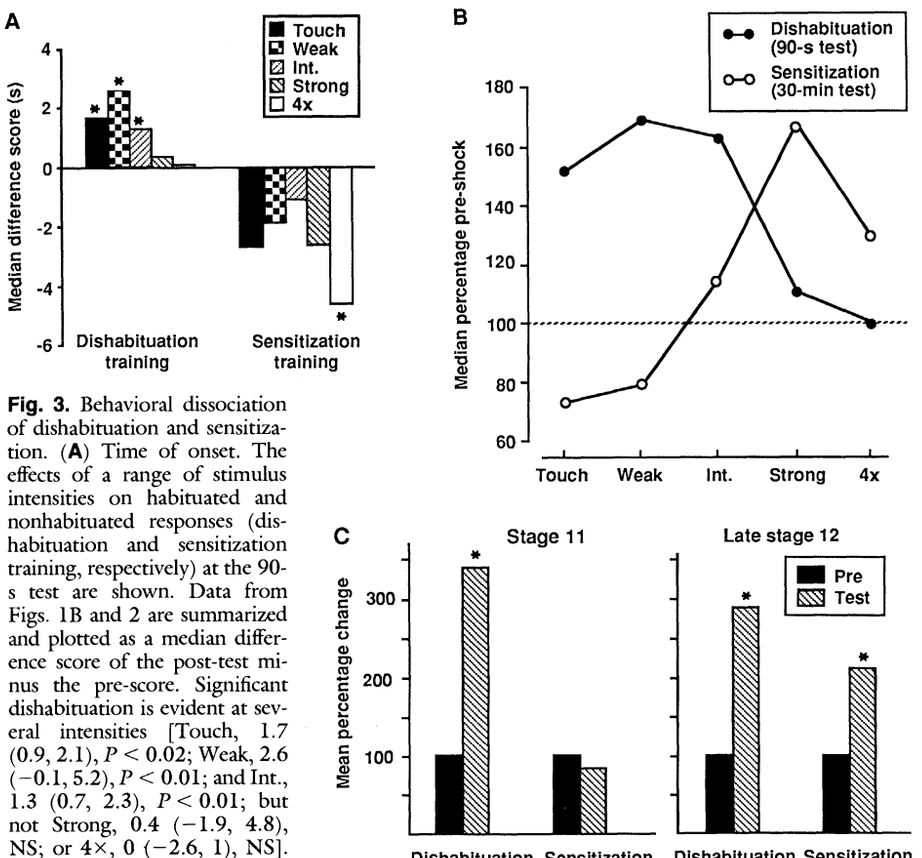

Fig. 3. Behavioral dissociation of dishabituation and sensitization. (**A**) Time of onset. The effects of a range of stimulus intensities on habituated and nonhabituated responses (dishabituation and sensitization training, respectively) at the 90-s test are shown. Data from Figs. 1B and 2 are summarized and plotted as a median difference score of the post-test minus the pre-score. Significant dishabituation is evident at several intensities [Touch, 1.7 (0.9, 2.1), $P < 0.02$; Weak, 2.6 (−0.1, 5.2), $P < 0.01$; and Int., 1.3 (0.7, 2.3), $P < 0.01$; but not Strong, 0.4 (−1.9, 4.8), NS; or 4×, 0 (−2.6, 1), NS]. In contrast, sensitization is not seen at any stimulus intensity; in all cases, tail stimulation produced suppression of nondecremented responses, with the strongest stimulus (4×) producing significant inhibition (Fig. 2). (**B**) Sensitivity to stimulus intensity. Reflex magnitude as a function of tail shock intensity for dishabituation and sensitization is summarized. The dashed line through 100% indicates baseline. Maximal dishabituation is produced by Weak stimuli (which produce no significant sensitization), and maximal sensitization is produced by Strong stimuli (which produce no significant dishabituation). (**C**) Developmental emergence. A summary of the development of dishabituation and sensitization is shown. Data from Rankin and Carew (*12*) have been reanalyzed. Data from juvenile stage 11 (20 days after metamorphosis) and from late stage 12 (90 days after metamorphosis) are shown. In stage 11, tail shock produces significant dishabituation ($n = 22$) [339.9% (119.2%, 489.5%), $P < 0.005$], but no sensitization ($n = 20$) [84.8% (58%, 115.7%), NS]; 10 weeks later in stage 12, tail shock still produces both significant dishabituation ($n = 10$) [290.7% (174.8%, 376.8%), $P < 0.005$] and significant sensitization ($n = 10$) [212.5% (104.7%, 311.3%), $P < 0.05$]. Test data are normalized to pre-shock levels.

REFERENCES AND NOTES

1. R. A. Rescorla and R. L. Solomon, *Psychol. Rev.* **74**, 151 (1967); N. J. Mackintosh, *The Psychology of Animal Learning* (Academic Press, New York, 1974).
2. J. W. Whitlow, Jr., *J. Exp. Psychol.* **104**, 189 (1975); ——— and A. R. Wagner, in *Habituation, Sensitization, and Behavior*, H. V. S. Peeke and L. Petrinovich, Eds. (Academic Press, New York, 1984), pp. 103–153.
3. B. Hochner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8410 (1986); *ibid.*, p. 8794.
4. P. M. Groves and R. F. Thompson, *Psychol. Rev.* **77**, 419 (1970); T. J. Carew, V. F. Castellucci, E. R. Kandel, *Int. J. Neurosci.* **2**, 79 (1971); T. J. Carew and E. R. Kandel, in *Synaptic Transmission and Neuronal Interaction*, M. V. L. Bennett, Ed. (Raven Press, New York, 1974), pp. 339–383.
5. T. J. Carew and C. L. Sahley, *Annu. Rev. Neurosci.* **9**, 435 (1986); R. D. Hawkins *et al.*, in *Handbook of Physiology: The Nervous System; Higher Functions of the Brain*, F. Plum, Ed. (American Physiological Society, Bethesda, MD, 1987) pp. 25–83; J. H. Byrne, *Physiol. Rev.* **67**, 329 (1987).
6. Five levels of tail stimulation were used. In ascending levels of intensity they were: Touch (brief contact with stimulating electrodes, no current); Weak, Int., and Strong (a single 1-s ac shock of 2.5, 50, and 100 mA, respectively, through bipolar

electrodes spanning the tail; and 4× (four 1-s shocks of 50 mA at a 1-s ISI).

7. Siphon withdrawal was elicted by a single 750-ms jet of seawater delivered by a waterpick. In all cases, the measure of response magnitude was the duration of siphon withdrawal [T. J. Carew, H. M. Pinsker, E. R. Kandel, *Science* **175**, 451 (1972)]. Response facilitation was computed by expressing the test score as a percentage of the "pre-score": for dishabituation the pre-score was the last response in the habituation series; for sensitization the pre-score was the mean of the two baseline (nondecremented) responses. All testing was carried out with blind procedures. Standard nonparametric statistics were used throughout. First, appropriate overall analyses of variance were carried out. Subsequent between-group comparisons were then made by means of Mann-Whitney *U* tests, and within-group comparisons by means of Wilcoxon signed rank tests. All probability values are two-tailed.
8. In all dishabituation groups, there was significant habituation before the delivery of the tail stimulus (Friedman analysis of variance, at least $P < 0.01$ in each case). There was no significant difference in habituation among the groups: Kruskal-Wallis between-group comparison of initial stimulus minus the mean of the last three stimuli in the habituation series ($H = 7.03$, NS).
9. There was a total of six groups of animals in the dishabituation series [a Recovery group, plus five stimulus intensities (*6*)]. Although only one Dishab. group (Weak) is illustrated in Fig. 1A, for all groups showing dishabituation, the results were the same. For the 90-s, 10- and 20-min tests, respectively, the results for each stimulus intensity, expressed as the median percentage (interquartile range in parentheses) were: Touch, 152% (127%, 173%); 335% (296%, 404%); and 395% (300%, 450%). Int., 163% (114%, 191%); 270% (184%, 1006%); and 270% (173%, 381%). Strong, 111% (66%, 296%); 191% (99%, 311%); and 231% (163%; 308%). 4×, 100% (68%, 115%); 227% (118%, 380%); and 188% (157%, 385%).
10. Earlier experiments by T. J. Carew, E. T. Walters, and E. R. Kandel [*J. Neurosci.* **1**, 1426 (1981)] examining classical conditioning of siphon withdrawal in *Aplysia* also suggested that sensitization might have delayed onset. A similar delayed onset of sensitization produced by foot shock has been observed in the startle reflex of the rat [J. M. Hitchcock and M. Davis, *Soc. Neurosci. Abstr.* **13**, 643 (1987)].
11. C. H. Rankin and T. J. Carew, *J. Neurosci.* 7, 133 (1987).
12. ———, *ibid.* **8**, 197 (1988).
13. T. G. Nolen and T. J. Carew, *ibid.*, p. 212.
14. Tail shock–induced inhibition has been observed in juvenile *Aplysia* (*11–13*) and may compete with the expression of dishabituation in juvenile animals in a manner similar to that shown in Fig. 3A [C. H. Rankin and T. J. Carew, *Soc. Neurosci. Abstr.* **13**, 816 (1987); *Behav. Neurosci.*, in press]. Moreover, a similar tail shock–induced inhibitory process in adult *Aplysia* has also been described by Mackey *et al.* (*17*) and by J. K. Krontiris-Litowitz, M. T. Erickson, and E. T. Walters [*Soc. Neurosci. Abstr.* **13**, 815 (1987)].
15. S. G. Rayport and J. S. Camardo, *J. Neurosci.* **4**, 2528 (1984).
16. K. J. Gingrich and J. H. Byrne, *Soc. Neurosci. Abstr.* **13**, 597 (1987).
17. S. L. Mackey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8730 (1987).
18. F. Belardetti, E. R. Kandel, S. A. Siegelbaum, *Nature* **325**, 153 (1987); D. Piomelli *et al.*, *ibid.* **328**, 38 (1987); D. A. Piomelli, E. Shapiro, S. J. Feinmark, J. H. Schwartz, *J. Neurosci.* 7, 3675 (1987).

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Poliovirus Host Range Is Determined by a Short Amino Acid Sequence in Neutralization Antigenic Site I

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The mouse-adapted strain of poliovirus type 2 (Lansing) induces fatal poliomyelitis in mice after intracerebral inoculation, whereas mice inoculated with poliovirus type 1 (Mahoney) show no signs of disease. Previous work indicated that the adaptation to mouse virulence is associated with the viral capsid proteins and that mutations in neutralization antigenic site I of poliovirus reduce neurovirulence of the Lansing strain in mice. The role of antigenic site I in mouse neurovirulence was further explored by constructing an antigenic hybrid virus. Six amino acids in antigenic site I of the Mahoney strain were replaced with a sequence specific for the Lansing strain by using a mutagenesis cartridge. The hybrid virus was neutralized by polyclonal antisera elicited by the type 1 and type 2 strains of poliovirus and by neutralizing monoclonal antibodies directed against antigenic site I of type 2 virus. The hybrid virus induced paralytic disease in mice, an observation demonstrating that a short sequence of amino acids in antigenic site I is an important determinant of poliovirus host range. Antigenic site I may be involved in attachment of poliovirus to cells of the mouse central nervous system.

TISSUE TROPISM OF VIRUSES AND the progression of viral disease in the infected host are determined by several factors. Specific cellular surface molecules that serve as viral receptors play an important role in these events. Another determinant is the genotype of a virus because it affects replication within the cell. Genetic variants of highly virulent viruses whose genomes differ by very few nucleotides may be dramatically attenuated even though adsorption and entry of the variant are not impaired.

Poliovirus, a member of the Picornaviridae, has served as a model system to study the molecular basis of viral pathogenesis, particularly the relationship of genotype and phenotype to neurovirulence and host range (*1*). Attenuation of poliovirus appears to be caused largely by mutations that impair viral replication within the cell (*2, 3*). Polioviruses, which occur in three serotypes, are human pathogens that can be propagated only in cultured cells of primate origin because other cell lines do not express a functional receptor molecule (*4*). Although most poliovirus strains can infect only primates, the Lansing strain of poliovirus type 2 [PV-2(L)] has been adapted to mice (*5*) and causes fatal paralytic disease when inoculated intracerebrally (*6*). In contrast, many other poliovirus strains, including type 1 (Mahoney) [PV-1(M)], although highly neurovirulent in primates including man, are avirulent in mice, even when administered in high doses (*7*). A molecular genetic analysis of PV-2(L) has revealed that the major determinant or determinants of the mouse-adapted phenotype are contained within the four capsid polypeptides (*7*). A study of the neurovirulence of neutralization escape mutants of PV-2(L) suggests that the mouse-adapted phenotype is determined by a specific region of VP1 located roughly between amino acids 90 and 105 (*8*).

The known chemical (*9*) and three-dimensional (*10*) structures of poliovirus, the results of analyses with neutralizing monoclonal antibodies (N-mAbs) and neutralization-resistant variants, and the information gained from immunizations with synthetic peptides led to the identification of three neutralization antigenic sites (N-Ag) of the poliovirion (*11*). One of these sites is a continuous sequence of amino acids (90–105) in VP1 and is identical with the region to which LaMonica *et al.* (*8*) had mapped the mouse-adapted phenotype of PV-2(L). We refer to this region as N-AgI. In the crystal structures of human rhinovirus 14 (*12*) and of PV-1(M) (*10*), N-AgI occurs as a loop near the apexes of the particle.

The development of infectious poliovirus cDNA clones (*13*) and transcription vectors that produce unlimited amounts of highly infectious RNA in vitro (*14*) have made possible the construction of various poliovirus recombinants useful for studying viral replication and pathogenesis. The method of "cartridge mutagenesis," which facilitates exchange of very small regions of the genome, was adapted for poliovirus (*15*). To

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