data indicate that, in man, there is no compensatory increase in the biosynthesis of testosterone under these conditions.

EMANUEL RUBIN
Department of Pathology, Mount Sinai School of Medicine, New York 10029
CHARLES S. LIEBER
Department of Medicine, Mount Sinai School of Medicine, and *Section of Liver Disease and Nutrition, Veterans Administration Hospital, Bronx, New York*
KURT ALTMAN
GARY G. GORDON, A. LOUIS SOUTHREN
Section of Endocrinology, New York Medical College, New York

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Experience Modifies the Plastic Properties of Identified Neurons

Abstract. *Crickets (*Acheta domesticus*) were reared in the presence of continuously repeating tone pulses. The responses of large abdominal interneurons to similar tone pulses were then compared. The giant interneurons of treated specimens are more resistant to habituation than those of control specimens.*

Exposure to sensory input during postnatal development can influence the nature of the vertebrate nervous system (*1*). If sensory input could be demonstrated to influence the development of the numerically simpler nervous system of an invertebrate, we might be able to determine, at the level of identified neurons, the mechanisms by which environmental modification of a nervous system occurs. We report here a successful modification of the response properties of identifiable interneurons by manipulation of sensory input during maturation. We found that interneurons exposed to continuous stimulation during postembryonic development habituate to that stimulus more slowly than do interneurons of unexposed specimens.

Our experiments were carried out on the abdominal giant interneurons of the cricket *Acheta domesticus*. These interneurons, which are the largest in the cricket nervous system, are excited by substrate vibrations detected by receptors located in the body wall and by sound and wind currents detected by mechanoreceptors located on paired abdominal sensory appendages, the cerci. The sensitivity to tones, which will be our major interest here, results from a monosynaptic input from sensory cells coupled to receptive hairs located on the cerci. The largest interneurons are sensitive to tones below 1000 hertz with peaks at approximately 100 and 500 hertz (*2*).

We reared crickets from hatching to adulthood in the presence of continually produced tone pulses of 500 hertz and 100 to 105 db (*3*). Tone pulses were 300 msec long and occurred at a rate of 1 to 2 per second. Control specimens were reared in identical cages in identical incubators but never heard the sound pulses. As the background noise level in these incubators was 80 db, we used a relatively high-intensity stimulus for treatment. In the first two experiments we reared 50 to 60 specimens randomly selected at hatching from two (and in the second experiment, four) females and tested 20 male specimens from these groups. In a third experiment we reared identical numbers of treated and control larvae from a single female and, in addition, exchanged the incubators weekly. All specimens were tested between 2 and 5 weeks after the adult molt. The treatment effects were identical in each experiment, although variability was reduced in the experiment where all specimens were siblings.

Table 1. Statistical analyses of habituation rates. The data for each specimen were fitted to a power curve of the form $Y = bX^m$. This curve was transformed to a straight line of the form $\ln Y = \ln b + (m) \ln X$. A *t*-test was then used to determine whether the slopes of control and treated groups were different. All the specimens in this experiment were male siblings. There were 14 specimens in the control group and 15 in the treated group. Abbreviations: c, control group; t, treated group; N.S., not significant.

Pulses per second	Constants		*t*	d.f.	*P*
	m	*b*			
0.5					
c	−.127	5.77	0.02	27	N.S.
t	−.097	5.64			
1.0					
c	−.334	6.78	−3.18	27	.01
t	−.176	6.16			
1.5					
c	−.422	6.47	−3.22	27	.01
t	−.231	5.36			
2.0					
c	−.794	7.12	−6.50	27	.001
t	−.303	5.17			
2.5					
c	−.944	6.97	−4.16	27	.001
t	−.433	4.37			

A phasic response consisting of six to eight action potentials was recorded at the onset of a tone pulse (Fig. 1A, upper trace). This extracellularly recorded activity was fed to a spike height discriminator. The threshold of the discriminator was set so that only the phasically driven large amplitude action potentials were counted (Fig. 1A, upper trace, dotted line). The action potentials above threshold were displayed as a series of dots on the face of a storage oscilloscope, which displayed the response decrement visually (Fig. 1B). Spike counts were obtained directly from this display.

The activity in a single interneuron accounts for most of the extracellular action potentials. Extracellular recording electrodes identical to those described above were placed in the usual position just anterior to the terminal ganglion and surrounded with Vaseline. A recording micropipette was then inserted in the soma of the medial giant interneuron (MGI) using methods described elsewhere (*4*). The neuron was anatomically identified by injecting the soma with Procion yellow or co-

balt chloride. Two examples of such simultaneous recordings are shown in Fig. 1C. Numerous trials in three different preparations demonstrated that 73 to 79 percent of the extracellularly recorded action potentials in response to a single tone pulse were recorded from a single neuron, the MGI.

The frequency response of the phasic interneurons was found to be identical for the control and treated groups (Fig. 1D). The frequency response was obtained by determining the number of spikes elicited by tone pulses of constant intensity (80 db) and 12 different frequencies arranged in random order with 5 minutes between trials. The standard errors of the mean for the treated and experimental groups overlap at every point. The curves are similar to those published previously (*2*). We conclude that long-term stimulation did not alter the frequency response of the giant interneurons.

However, when habituation rates of the two groups were compared, it was found that treated specimens were more resistant to habituation than control specimens (Fig. 1E). Habituation rates were determined in the usual manner; the specimen was stimulated with short tone pulses (500 hertz, 80 db, 300-msec duration) repeated at 0.25 to 2.5 times per second; each animal was tested at six tone repetition rates with a 10-minute rest between trials. The number of spikes per tone pulse in a train was determined (Fig. 1B) and plotted as a function of the pulse number in the train (Fig. 1E). Power functions best describe the data, giving correlation coefficients greater than .90 for all data sets (*5*). When repetition rates are higher than 0.5 pulse per second, the giant interneurons of treated specimens habituate more slowly than control specimens (Table 1). At lower repetition rates, habituation occurs slowly, if at all, and there were thus no detectable differences between the two groups.

The apparent alteration in the transmission of sensory information might reflect a change in the sensitivity of the receptors or a generalized change in the reactivity of the animals. However, the results from both the frequency response tests (Fig. 1D) and the initial test during habituation (Fig. 1E) show no significant difference in the effectiveness of the stimuli; in fact, the response of the treated group is slightly smaller in both cases (*6*).

We deliberately used an intensive and long-term treatment in order to increase the possibility of inducing a change. We have not yet investigated the minimal conditions needed for the effect; by analogy with other systems there may be a critical period for the change (*7*). However, we have been able to show that short-term (1-hour) treatments of adult animals produce

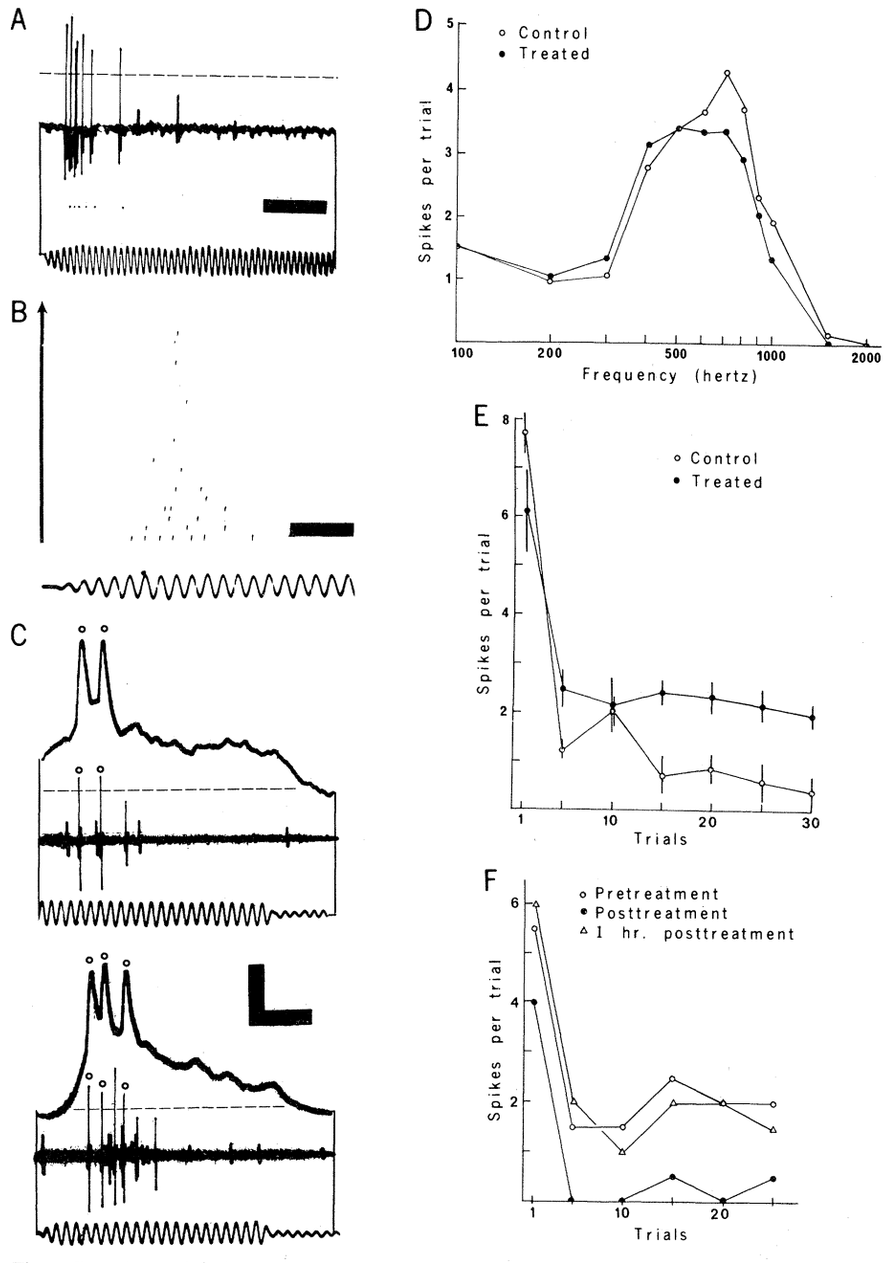

Fig. 1. Experimental design and results. (A) Sample recording. Upper trace, extracellular recording from one connective just anterior to the last abdominal ganglion. The dashed line indicates threshold level of spike height discriminator. Middle trace, dots representing output of the spike height discriminator. The threshold level of the discriminator was set just above all spontaneous activity. Lower trace, tone pulse of 500 hertz and 80 db. Horizontal calibration, 20 msec. (B) Display of spikes, indicated by dots from the spike height discriminator, in response to 25 tone pulses repeated at a rate of 2 per second. The spikes in response to the first tone pulse are in the row nearest the tone pulse and subsequent responses are farther from the tone, which is indicated by the arrow at the left. Horizontal calibration, 10 msec. (C) Simultaneous intracellular and extracellular recordings. The upper trace in each panel is the intracellular record; the middle trace is the extracellular recording from the connective anterior to the abdominal ganglion; the lower trace represents the tone pulse. Correlated MGI action potentials in the intracellular and extracellular records are indicated by circles. Dashed line is as in (A). Vertical calibration for the top traces only, 4 mv. Horizontal calibration, 20 msec. (D) Frequency response curve for the MGI and lateral giant interneuron (LGI) of control and treated specimens. The treated specimens were exposed to 300-msec tones repeated twice per second for the entire postembryonic period of development. Each point represents the mean for nine animals. The standard errors overlap at every point. (E) Habituation rates of control and treated specimens. The treatment was as in (C). Vertical bars indicate S.E.M. The curves are significantly different at the .001 level (Table 1). (F) Habituation rates after a treatment of 1 hour. Treatment tones were equivalent to those used in the long-term experiment (100 db, repeated at a rate of 2 per second). ○ — ○, Habituation rate tested just before the treatment (mean of two tests); ● — ●, habituation rate obtained 2 minutes after the treatment; △ — △, rate obtained 1 hour after the treatment ended.

no effect on transmission tested an hour later, and, as expected (*8*), actually increase habituation when animals are tested immediately after treatment. The experimental design of these short-term experiments consisted of selecting a specimen at random from the stock population, mounting it in the usual manner, testing the habituation rate 1 hour after mounting, then exposing the specimen to intense (100 db) tone pulses for 1 hour, retesting the habituation rate immediately after the treatment, and finally, allowing a 1-hour recovery period and testing again. Individual specimens exhibit increased habituation when tested 2 minutes after treatment (Fig. 1E), but recovery from this short-term depression occurs in 1 hour. Thus, short-term effects are unlikely to influence our results.

Our conclusion is that long-term use of the pathways between the afferent neuron and the giant interneurons during maturation leads to the development of a pathway that is less plastic. We do not know how permanent the effect is because we have not yet systematically tested at long intervals after removing treated specimens from the stimulus.

It is significant that the change was not a mere increase or decrease in efficacy; no difference was detected when single stimuli were used. Instead, the lability of the system was altered: the habituation curve was not shifted, but its slope was changed.

We have no evidence as yet about the site of the change, but this is a monosynaptic pathway and the postsynaptic cells are accessible for intracellular recording. Hence we feel that this preparation is an excellent candidate for the cellular analysis of a developmental change.

R. K. MURPHEY, S. G. MATSUMOTO
Department of Biology, State University of New York, Albany 12222

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Consequences of a Nationwide Ban on Spray Adhesives Alleged to Be Human Teratogens and Mutagens

Abstract. *A report of an association of chromosome breakage and birth defects with spray adhesive exposure resulted in a ban on the sale of these products and nationwide publicity warning exposed women. Six months later the ban was removed; the association could not be confirmed. Replies to questionnaires sent to medical genetics centers throughout the United States revealed that more than 1100 inquiries had been received and more than 1200 working days were expended because of the issue. Eleven exposed women underwent diagnostic amniocentesis, and one elected to abort her fetus. Eight other women who were exposed also elected to do so, but without first undergoing diagnostic amniocentesis. The episode illustrates some of the unexpected and unnecessary consequences that can arise from the false identification of an environmental agent as a mutagen or teratogen.*

In August 1973, the United States Consumer Product Safety Commission announced a reported association between exposure to some spray adhesives and chromosomal breakage and birth defects. The sale of these products was abruptly banned, and they were recalled from the market (*1, 2*). The Commission widely publicized a warning to all those exposed, particularly pregnant women, and urged them to consult a physician concerning chromosome studies. The ban was withdrawn in 6 months because the purported associations could not be confirmed, and no toxicity of the substances in question could be demonstrated. In fact, the results of reexamination of the original slides by other investigators did not confirm the first interpretation of increased chromosome breakage in those exposed (*3*).

The medical subspecialists for exposed individuals heeding the Commission's advice were primarily the medical geneticists, especially those doing genetic counseling and providing services in cytogenetics. Some centers were reported to have been deluged for requests for counseling and diagnostic services as a result of the Commission's announcements (*1*).

In an attempt to estimate the minimum impact of this episode, we sent questionnaires in May 1974 to all individuals in the United States listed in a national directory (*4*) as providing services both in diagnostic cytogenetics and genetic counseling (Table 1). They were asked to estimate the number of inquiries received, the number of chromosome studies of those who had made inquiries, the total number of working days expended because of this episode, the number of amniocenteses performed, the number of induced abortions, and any other adverse outcomes. They were also asked to comment on any possible beneficial consequences of this episode for those exposed.

There were 190 replies from independent units of which 182 were from active independent centers (Table 1) (*5*). There was a great range in the number of reported inquiries at these centers. More than one-third of the centers reported no inquiries

Table 1. Response categories to questionnaire and inquiries reported by those independent active centers which replied.

Active independent centers reporting	
No inquiries	52
1 to 5 inquiries	68
6 to 10 inquiries	31
11 to 15 inquiries	8
16 to 20 inquiries	8
21 to 25 inquiries	2
> 25 inquiries	7
"Some" inquiries	6
Subtotal	182*
Individuals with collaborative arrangement†	40
Inactive independent centers	8
No reply	5
Total	235

*At the 176 centers reporting an exact number of inquiries, the mean was 6.81 queries. The maximum number of queries reported was 200. The 25, 50 (median), and 75 percent quartile boundaries were 0, 3.2, and 7.6, respectively. The (minimum) total number of queries at these 176 centers was 1198. †Individuals with a separate listing in the directory who are affiliated with an individual at an active independent center from whom a reply was received. See also (*5*).

Table 2. Frequency of chromosome studies at 130 active independent centers receiving inquiries concerning spray adhesives.

Frequency of studies	Centers (No.)
0	49
1 to 5	58
6 to 10	13
11 to 15	4
16 to 20	2
21 to 25	1
> 25	1
"Some"	2*
Total	130
Range	0 to 44
Mean* †	2.97
Quartiles*	
25 percent	0
50 percent (median)	2.0
75 percent	4.2
Total studies (minimum)‡	380

*Those replying "some" were excluded from calculations of mean, quartiles, and minimum total studies. †Standard deviations were not calculated because of the observed skewed distribution. ‡At centers providing exact estimates.