second polymer is able to join the first polymer at the cellular level, even if it is added more than 1 day after the first polymer [for example, poly(rC) applied to RK 13 cell cultures 28 hours after poly(rI)] (Table 1). According to the second alternative, the polymer added first might exert an effect on the cell that is required for the antiviral activity of the second polymer.

The fact that separate administration of the individual homopolymers only partially restores the interferon inducing capacity of poly(rI) • poly(rC) in vivo, does not necessarily refute the hypothesis of a two-step action mechanism of the homopolymers. When injected separately in the whole animal, the two homopolymers are probably going to different cells and do not interact with the same cell, as they do in vitro. Hence, the partial antiviral effect obtained with poly(rI) and poly(rC) in vivo, when injected in rapid succession, may be ascribed to an association of the two homopolymers and to activity of the double-stranded complex.

The finding that the antiviral activity of the poly(rI) • poly(rC) complex in vitro can be equaled and even surpassed by successive administration of the component homopolymers may be an important step in the study of the mechanism of interferon production by nucleic acids of either synthetic or viral origin, and might help in identifying the ultimate trigger site for interferon production.

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8. We thank Miss A. Van Lierde for technical assistance. E.D.C. is "Aangesteld navorser" of the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek.

20 April 1971

## Habituation of Electrically Induced Readiness to Gnaw

Abstract. *Electrical stimulation of the hypothalamus in prairie dogs* (Cynomys ludovicianus) *produced a readiness to gnaw which decreased over time, exhibited spontaneous recovery, and could be dishabituated by foot shock. The response decrement was in part habituatory and could modify the interaction between a stimulation-induced readiness to gnaw and a physiologically induced hunger. Functional plasticity of stimulation-induced behavior might be accounted for, in part, by habituation.*

Electrical stimulation of the hypothalamus has been shown to produce a *readiness* for such behavior as eating, drinking, gnawing, nesting, attacking, and mating (*1*). The term "readiness" has been used because the stimulation-induced behavior is dependent upon access to appropriate goal objects (*2*). Valenstein, Cox, and Kakolewski (*3*) have described "a procedure for modifying behavior elicited by hypothalamic stimulation" in rats. This procedure first involved stimulating a rat in the presence of food, water, and gnawing material. The preferred goal object (for example, food) was then removed and the subject was repeatedly stimulated in the presence of the remaining goal objects.

After several nights of such "training" the subject was again stimulated in the presence of all three goal objects. When this procedure was used, significant preference changes were reported (for example, an animal that ate on a pretraining test drank or gnawed on the posttraining test). Valenstein *et al.* seemed to assume that the preference changes resulted primarily from environmental manipulations, that is, removal of the preferred goal object, during stimulation rather than the continued stimulation per se. But this assumption does not explain how a long series of stimulation periods with *all three* goal objects present changed the preference of several animals (*4*). Furthermore, these investigators did not include a control group stimulated in the absence of any goal object nor did they report any attempt to retrain an animals to its initial preference.

Questioning the assumption that environmental manipulations influence the preference changes, Wise (*5*) has advanced the alternative hypothesis that continued stimulation can reduce the threshold for an initially latent response to the extent that this response can compete with behavior shown to be prepotent on initial tests. A third possible explanation for the preference changes reported by Valenstein *et al.* is that prepotent behavior observed on the first test habituated (*6*) more rapidly than other initially latent tendencies. For example, if stimulation-induced hunger were prepotent on the initial test but habituated more rapidly than stimulation-induced thirst, we would expect the animal would eat when first stimulated but would, at some time, switch to drinking if stimulation continued long enough. The acceptability of such an explanation rests upon the assumption that a stimulation-induced readiness can habituate. Habituation of a variety of peripherally evoked behaviors has been demonstrated (*7*). Habituation of cortical and behavioral arousal elicited by intracranial stimulation has also been shown (*8*). We have attempted to demonstrate the habituation of a stimulation-induced readiness to gnaw and to determine how this habituation alters the interaction between gnawing and other motivational tendencies.

Bipolar electrodes made of Teflon-coated wire 0.20 mm in diameter, with insulation removed 0.4 mm from the tips, were implanted in the lateral hypothalamus (*9*) of 13 adult black-tailed prairie dogs (*Cynomys ludovicianus*). Six of these animals showing reliable gnawing on several daily stimulation tests were selected for study.

Each subject was placed in a 34 by 25 by 66 cm test chamber containing 16 loose white pine blocks (7.0 by 1.5 by 2.0 cm). (Foam rubber blocks of the same size were used in place of wood blocks for one animal with defective teeth.) Animals were adapted to the box for 10 minutes; none showed spontaneous gnawing. To determine each animal's gnawing threshold, continuous stimulation (*10*) was administered at 1.0 volt and then increased at a rate of 3 volt/min. When gnawing began, stimulation was turned off. The subject was then given a series of 30-second stimulation trials with 3 minutes between trials. On the first trial the voltage was set at 0.5 volt below threshold and increased in 0.2-volt steps on subsequent

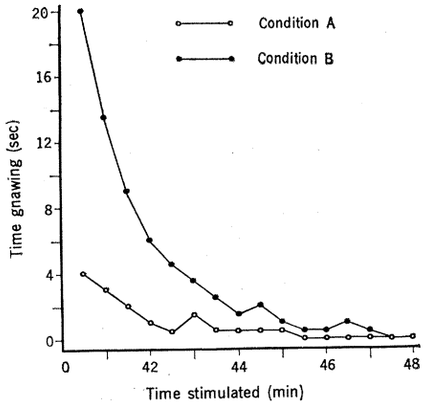


Fig. 1. Response decrement curves for the first 8 minutes of testing after two treatment conditions in experiment 2. Testing consisted of administering stimulation for 40 minutes with gnawing blocks available. Prior to testing under condition A, each animal received 40 minutes of stimulation while restrained from gnawing; no prior stimulation was administered for condition B. Each point represents the mean score for six animals.

trials until the animals gnawed 15 or more seconds during a 30-second trial. The amperage of this "criterion" trial was used for that animal during the remainder of the experiments. Both current and voltage were monitored on an oscilloscope. Gnawing was recorded by an observer pressing a microswitch. The microswitch activated a 0.5-second counter which printed out every 30 seconds.

The first experiment was carried out on three consecutive days. On day 1 each animal was stimulated continuously at its criterion amperage until less than 2 seconds of gnawing occurred during a 30-second interval. Gnawing blocks were then removed and the stimulation continued during a "rest period" of 30 minutes. Stimulation then ceased for 1 minute while gnawing blocks were replaced, and then was readministered for an additional 30 minutes.

Day 2 differed from day 1 in that, after the 2-second criterion had been reached, the stimulation was turned off for 31 minutes during the rest period. Blocks were removed and replaced as on day 1. Stimulation was then resumed for 30 minutes. Day 3 procedures were identical to those of day 1 and were included to insure that there were no pronounced order effects among the days.

Although stimulation parameters remained constant, gnawing declined steadily during stimulation. Thirty minutes of rest *with* stimulation on days 1 and 3 produced less average recovery than 30 minutes of rest *without* stimulation on day 2 ($F = 8.64$, d.f. $= 1/25$, $P < .01$).

If the decrement shown during the prerest period were totally *response-induced* (for example, produced by muscular fatigue or soreness of mouth), 30 minutes rest *with* stimulation should have been as effective in restoring the readiness to gnaw as 30 minutes rest without stimulation. This finding indicated that the present decrement was at least in part habituatory.

The results of the first experiment left open the possibility that the prerest decrement could have been *exclusively* habituatory with no response-induced decrements involved, since conceivably 1 minute without stimulation (days 1 and 3) could have been almost as effective in dishabituating the gnawing as was 31 minutes without stimulation (day 2). Therefore, a second experiment was conducted to determine whether or not the response decline as shown with continuous stimulation in the first experiment could be partly response-induced. Two treatment conditions were administered in counterbalanced order. For condition A, each subject received 80 minutes of continuous stimulation. During the first 40 minutes of stimulation a nylon cord suspended from above the chamber and attached to the skull connector kept the subject from reaching the blocks on the floor but permitted free movement about the chamber. During the last 40 minutes the cord was removed and the animal was free to gnaw. For condition B, the same procedure was followed except that no stimulation was administered during the first 40 minutes.

If habituation were the only factor producing the observed response gradients (Fig. 1), gnawing after 40 minutes of stimulation (condition A) would not be expected, since no gnawing occurred after 8 minutes of stimulation under condition B. Apparently the observed gradients were partially response-induced. However, 40 minutes of stimulation prior to head release reduced subsequent gnawing by 24 percent, indicating that habituation to brain stimulation occurred even in the absence of gnawing. Total gnawing averaged across animals was 16 seconds for condition A and 66 seconds for condition B ($t = 8.01$, $P < .01$, correlated $t$-test). This again demonstrated that stimulation-induced gnawing can habituate.

Since recent evidence indicates that habituation and dishabituation may be independent processes (*11*), it is conceivable that a habituating response could not be dishabituated. However, since investigators have traditionally used dishabituation as one of the criteria for a habituating response (*12*), we believe that demonstrating dishabituation would add further support to the hypothesis that at least part of the decline in stimulation-induced gnawing was due to habituation rather than simple fatigue or receptor adaptation. Each subject was placed in the test chamber and given a 3-second, 2-ma foot shock to be certain that foot shock alone did not produce gnawing. Intracranial stimulation was then administered continuously and gnawing was recorded. When an animal's gnawing declined to the criterion of less than 2 seconds per 30-second interval the foot shock was again administered. Gnawing which resumed after foot shock was again allowed to decay to criterion, at which time foot shock was readministered. This procedure was repeated until the subject no

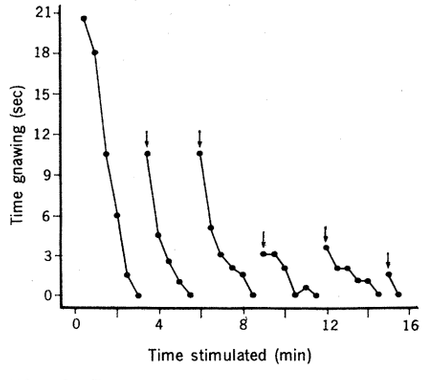


Fig. 2. Response decrement curves after successive foot shocks in experiment 3. The arrows indicate when a 2-ma, 3-second foot shock was applied. Each point represents the mean score for six animals.

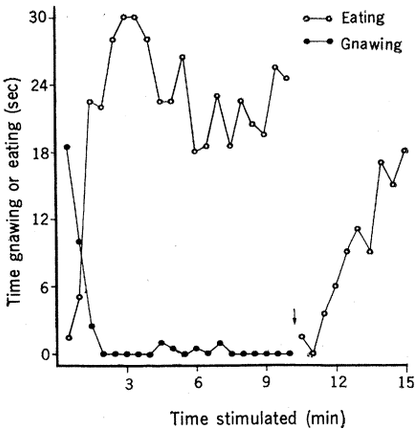


Fig. 3. Time spent in gnawing or eating. The arrow indicates when simulation was turned off. Each point represents the mean score for five animals.

longer responded to the dishabituatory stimulus.

The ability of the foot shock to repeatedly dishabituate gnawing was demonstrated (Fig. 2). In accordance with the classical model of habituation (*12*), foot shock gradually lost its dishabituatory power, that is, "habituation of dishabituation" occurred.

We next wished to determine whether the habituatory decrement demonstrated in the first three experiments would modify the interaction between stimulation-induced readiness to gnaw and physiologically induced hunger. Subjects were totally deprived of food for 6 days. On the seventh and eighth days of deprivation each subject was placed in the test chamber and allowed to eat from a dish of wet mash for 30 seconds. On day 1 of experiment 4 (the ninth day of deprivation) each subject was stimulated for 10 minutes in the test chamber with gnawing blocks available. On day 2 the food dish was also introduced and, after the subject had eaten for 15 seconds, stimulation was administered for 10 minutes. One observer operated microswitches to record time eating and time gnawing; another observer recorded latencies for (i) gnawing after stimulation began, (ii) returning to eat during stimulation, and (iii) returning to eat after stimulation ceased. Day 3 procedures were identical to those of day 1.

Less gnawing occurred when food was present (day 2) than when food was absent (average of days 1 and 3) (correlated $t = 2.79$, $P < .05$). On day 2 five animals (*13*) left the food dish and began to gnaw within 4.5 seconds (mean = 3.3 seconds) after stimulation began (Fig. 3). These animals returned to the mash and resumed eating within 82 seconds (mean = 60.4 seconds) after onset of stimulation. Four subjects never gnawed after they resumed eating. One animal vascillated between gnawing and eating but devoted the last 180 seconds of the stimulation interval exclusively to eating. These results suggest that at the beginning of the stimulation interval the stimulation-induced readiness to gnaw was stronger than the physiologically induced hunger, but as habituation progressed the tendency to gnaw could not compete with the more stable physiologically induced motive.

An unexpected finding was that animals stopped eating upon termination of stimulation. One subject did not resume eating during the 15 minutes he was left in the test chamber after stimulation. The other four subjects resumed eating between 77 and 271 seconds after stimulation. This poststimulation inhibition of eating could be explained by assuming that, along with inducing a readiness to gnaw, the stimulation induced a readiness to eat (stimulation-induced hunger) which summated with physiological hunger. Total hunger might then habituate, as did the readiness to gnaw. In this situation, stopping the stimulation might further reduce hunger to below threshold level.

The results of these four experiments indicate that a stimulation-induced readiness can habituate and that this habituation can alter the interactions between competing motivational tendencies. In light of these results, previous evidence for the plasticity of stimulation-induced behaviors might be reevaluated. If hypothalamic stimulation can simultaneously elicit two behavioral tendencies, the behavior manifested may depend upon which of two types of goal objects are available. When both types are available the manifest behavior may depend upon which readiness is prepotent. If the initially prepotent readiness habituates, another behavior with a flatter habituation gradient may become manifest independent of environmental manipulations. For example, an animal may show preference for eating over drinking when first stimulated. But a change in preference after long periods of stimulation may not depend upon a conditioned preference for either food or water but may result from a differential habituation of stimulation-induced hunger and stimulation-induced thirst. We do not question the functional plasticity of neural mechanisms underlying hypothalamically controlled behavior, but wish only to emphasize that there are several processes which may produce alteration of these mechanisms, for example, habituation, sensitization, classical conditioning, and instrumental conditioning. The relative importance of these processes in modifying the behavioral effects of intracranial stimulation is still to be determined by carefully controlled investigations.

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9. All points used in this study were within 1 mm of a point A15.5, V0.0, L2.8 in the atlas by R. H. Carlson and J. N. Kott [*Acta Anat.* **77**, 321 (1970)].
10. In order to minimize polarization of electrodes, all stimulation was carried out with a wave form devised by J. C. Lilly, G. M. Austin, W. W. Chambers [*J. Neurophysiol.* **15**, 319 (1952)].
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13. In the wild, prairie dogs spend considerable time firming up the walls of their burrows and the mounds at the opening of their burrows by a species-characteristic response called nose tapping. Nose tapping can sometimes be produced by electrical stimulation if wet dirt is available to the animal. In experiment 4, one of the subjects stopped eating and began nose tapping rather than gnawing when stimulation was administered. The data from this animal are omitted from the results summarized in Fig. 3.
14. We thank Verlis L. Setne for help in preparing the illustrations and Bartlett D. Moore III for technical assistance. Supported in part by NIMH grant No. 1 RO3 MH17236-01 MSH and by NIMH predoctoral fellowship No. 5 501 MH45273-02 awarded to R.E.C.

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## Agglutinin Interaction with Embryonic and Adult Cell Surfaces

Concerning the agglutinability of certain chick embryo cells by concanavalin A (Con A) and wheat germ agglutinin (WGA), Moscona (*1*) states that the previous experiments of Burger and Pollack and Burger (*2*) and Inbar and Sachs (*3*) were carried out with "adult cells from established cell culture lines." This statement is not completely consistent with the experimental facts. A substantial portion of these studies was carried out with the 3T3 line of cells, which was derived from a Swiss mouse embryonic culture (*4*), and with its virus-transformed variants. Thus, the failure of Con A or WGA