lines. The linear arrays of HRP patches have dimensions similar to the deoxyglucose stripes (a repeat distance of approximately 500 μm) (*11*), and both patterns seem to follow a generally posteromedial direction from the lateral border between areas 17 and 18. The possible correspondence of the two systems raises interesting questions. Perhaps the HRP pattern, for example, constitutes an anatomical substrate for the orientation-selective system. In this instance, what appears as a strict morphological periodicity may in fact subserve a functional continuum, with orientation determined by the degree to which single neurons enter into the widely connected system. Alternatively, the deoxyglucose columns may not actually reflect the orientation system that has been identified physiologically. In particular, can we be sure that during vertical stripe stimulation, the columns correspond to groups of vertical-selective neurons, and the nonlabeled interspaces correspond to horizontally selective neurons? In the same tissue, therefore, stimulation with horizontal stripes should result in an exact mirror reversal of the labeled and nonlabeled spaces. In combined physiological and deoxyglucose experiments in cats, lesions marking the locus of vertical selectivity apparently corresponded to peaks of verticality induced deoxyglucose patterns (*14*), but no direct evidence is yet available to confirm the phase shifting of the deoxyglucose pattern after stimulation with horizontal as opposed to vertical lines. As the HRP pattern in the tree shrew appears to illustrate a fixed anatomical locus, its correspondence with the pattern produced by deoxyglucose orientation suggests that the deoxyglucose pattern is also fixed and thus might not be directly related to the orientation aspect of the stimulus.

How literally should we take the image of a cortical column as a functional entity, designed to segregate out different submodalities? We might rather entertain the view of multiple repetitive structures intrinsic to the cortex, which may be adapted to fulfill a variety of functions. It may be useful to consider the vertical radial component of the cortex as enmeshed in an elaborate horizontal laminar organization, possibly in a multiple lattice-like design. Although the influence of each lattice could be translated through the cortical depth, this concept stresses the primacy of a particular lamina in elaborating a given function and underlines the importance of dynamic interconnections throughout the horizontal as well as the vertical extent of the cortex.

Finally, we have found (*15*) that similarly organized intrinsic connections occur in other species and may thus constitute a basic feature of neocortical structure. The relation between this anatomical framework and other reported periodicities remains to be established.

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Behaviorally Conditioned Immunosuppression and Murine Systemic Lupus Erythematosus

Abstract. *Development of autoimmune disease in female New Zealand hybrid mice was dramatically modified by classical conditioning of immunosuppression. Groups of animals received each week a solution of sodium saccharin (conditioned stimulus). One group of conditioned animals received an injection of cyclophosphamide (the unconditioned stimulus) after half of the weekly occasions when they received the saccharin solution. The rate of development of proteinuria and mortality were significantly retarded in these conditioned mice relative to untreated controls and nonconditioned animals that received unpaired treatment with saccharin and cyclophosphamide.*

Several converging lines of evidence implicate the central nervous system in the regulation of immune processes (*1*). Of relevance here is that behavioral conditioning procedures can be used to suppress humoral and cell-mediated immune responses (*2*). Conditioned immunosuppression is accomplished by pairing consumption of a novel drinking solution—the conditioned stimulus (CS)—with an immunosuppressive drug, for example, cyclophosphamide—the unconditioned stimulus (US). When subsequently treated with antigen, conditioned animals that are reexposed to the CS show attenuated immune responses. The present study was designed to examine the impact of conditioned immunosuppression on the development of systemic lupus erythematosus (SLE) in New Zealand mice, an autoimmune disease for which the female (NZB × NZW)F_1 (NZF_1) mouse has become a standard experimental model (*3*). Treatment of NZF_1 mice with cyclophosphamide prolongs survival of animals that would otherwise develop a lethal glomerulonephritis between approximately 8 and 14 months of age (*4*). On the basis of observations that immune responses can be suppressed by conditioning procedures, we hypothesized that, in conditioned mice, the substitution of conditioned stimuli (placebo treatment) for the immunosuppressive drug would delay the development of proteinuria and mortality relative to nonconditioned animals treated with the same dose of drug.

Individually caged female NZF_1 mice were maintained under a 12-hour light-dark cycle and given free access to food and water. A chemotherapeutic regimen was initiated when the animals were 4 months of age. Once each week for 8 weeks all the animals were administered a 0.15 percent solution of sodium saccharin (SAC) by pipette (up to 1.0 ml). Cyclophosphamide (*5*) was injected according to the following schedule.

The standard treatment group (C100) received, once weekly, an intraperitoneal injection of cyclophosphamide (30 mg/kg) immediately after they received the SAC solution. These stimuli were presented at the same time on the same day of each week. Results derived from this

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conditioned group defined the effects of traditional immunosuppressive therapy administered for 8 weeks. The dosage and duration of treatment prolonged survival but was insufficient to prevent the ultimate development of SLE.

Another conditioned group (C50) received, two times (in random sequence) in each 4 weeks, an intraperitoneal injection of cyclophosphamide (30 mg/kg) after they received the SAC solution. For the two times in each 4 weeks that they did not receive the drug they received an intraperitoneal injection of saline following SAC.

A nonconditioned group (NC50) also received cyclophosphamide injections after the SAC presentations two times in each 4 weeks, but these stimuli were administered on a noncontingent basis (that is, on different days of the same week).

Control animals received no immunosuppressive therapy. They did, however, receive the weekly dose of SAC solution and injections of saline on a noncontingent basis.

Group NC50, since they received only 50 percent of the cyclophosphamide that group C100 received, were expected to manifest symptoms of SLE and die sooner than animals treated weekly. Group C50 also received only 50 percent of the drug given to group C100. However, to the extent that reexposure to SAC (the CS paired with cyclophosphamide) is capable of eliciting a conditioned immunosuppressive response, it was predicted that the animals in group C50 would show a greater resistance to the development of SLE than the animals in group NC50.

At weekly intervals, proteinuria was measured on freshly expressed urine samples with tetrabromphenol paper ("Albustix") (*6*). When autolysis was not extreme or when moribund animals were killed, glomerulonephritis was examined histologically (and, in all instances, verified). Ten animals died without showing proteinuria and were eliminated from the experiment.

As expected, the weekly cyclophosphamide treatment delayed the onset of proteinuria and prolonged the survival of NZF_1 mice in group C100. Considering the total population of animals that developed SLE, there was a significant difference in the onset of proteinuria (values consistently ≥ 100 mg/100 ml) [$F(3, 96) = 8.28$; $P < .001$]. Since the mice in all groups were likely to develop proteinuria and die, the longer the disease was monitored the less likely it would be to discern treatment effects. Differences among the groups (Fig. 1A) become

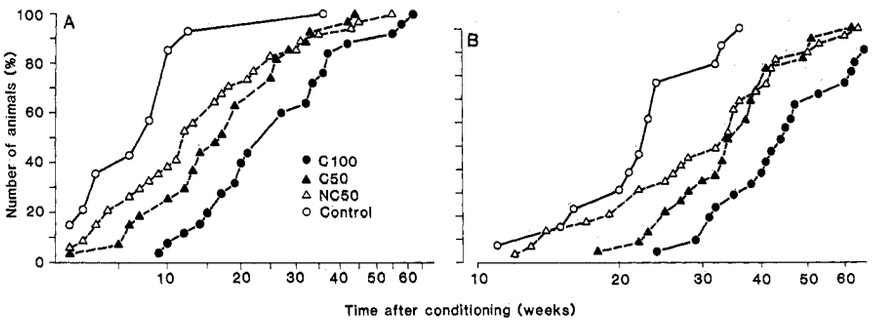

Fig. 1. (A) Rate of development of an unremitting proteinuria in NZF_1 female mice under different chemotherapeutic regimens. Group C100 ($N = 25$) received weekly a saccharin drinking solution (SAC) followed by an injection of cyclophosphamide (30 mg/kg); group C50 ($N = 27$) received weekly the SAC followed by an injection of cyclophosphamide (30 mg/kg) two times in each 4 weeks or an injection of saline; group NC50 ($N = 34$) received weekly the SAC and an injection of cyclophosphamide (two times in each 4 weeks) but the SAC and the drug were not paired; control mice ($N = 14$) received SAC weekly but no cyclophosphamide. (B) Cumulative mortality rate in NZF_1 female mice maintained on different chemotherapeutic regimens. Symbols as in (A).

striking when one uses as a reference point the rate of development of proteinuria for the initial 50 percent of the population developing the disease [$F(3, 47) = 18.29$; $P < .001$]. Animals in group C100 developed proteinuria later than any of the other groups ($P < .001$ in each instance). The nonconditioned animals (group NC50) did not differ from the untreated controls ($t = 1.78$), whereas the conditioned animals in group C50 developed proteinuria significantly more slowly than the untreated controls ($t = 3.86$, $P < .001$). The critical comparison between groups C50 and NC50 revealed that group C50 developed proteinuria significantly more slowly than group NC50 ($t = 2.38$, $P < .05$).

There were also group differences in mortality [$F(3, 85) = 10.49$; $P < .001$] that were especially dramatic when one considers the rate at which the first half of each group died [$F(3, 44) = 15.67$; $P < .001$] (Fig. 1B). Nonconditioned animals (group NC50) did not differ from untreated controls ($t = 1.62$). In contrast, group C50 survived significantly longer than untreated controls ($t = 4.24$, $P < .001$) and did not differ statistically from group C100 ($t = 1.28$), animals that received twice as much drug. Again, the critical comparison is between groups C50 and NC50, which received the same amount of drug: group C50 survived significantly longer (27.6 ± 1.5 weeks) than group NC50 (22.1 ± 1.7 weeks) ($t = 2.42$, $P < .05$).

Differences in both the rate of development of proteinuria and mortality can be traced to the variable onset of disease among the groups. Within-group correlations between the development of proteinuria and mortality ranged from .66 to .84 and were statistically significant in all instances. The interval between the development of proteinuria and death was also relatively constant (ranging from 14.4 ± 1.2 to 16.7 ± 1.2 weeks). Therefore, although the progression of SLE followed a similar course in all groups, there was a clear difference in the onset of disease that could be attributed to the differential treatment of the groups. These differences were consistent with the effects of cyclophosphamide in retarding the development of SLE. The results were also consistent with previous observations of conditioned immunosuppression (*2*) and with predictions that follow from the application of such conditioning within this biologic model.

The mechanisms mediating these conditioning effects are unknown. There are, however, several possibilities. An elevation in adrenocortical steroid levels, for example, might be invoked to explain the observed differences. Novel stimuli (saccharin) can elicit an adrenocortical response, as can an injection of saline or cyclophosphamide (*7*). Conditioned and nonconditioned animals, however, received the same number of such stimuli. Moreover, since combined environmental stimuli are not additive (*8*), it could be argued that the nonconditioned animals (group NC50) exposed to SAC and intraperitoneal injections at different times received twice as many "stressful" experiences as the conditioned animals (group C50). A differential adrenal response (that is, a conditioned elevation in corticosterone level) is possible but unlikely. It is possible to condition an elevation in steroid level in fluid-deprived rats (*7*), but 30 mg of cyclophosphamide is insufficient to induce an aversion to a flavored drinking solution in NZF_1 or C57BL/6 mice (*9*).

Several other endocrine- and neuroendocrine-immune system interactions have been described (*1*), and these physiologic processes may be subject to or influenced by conditioning.

The present study does not provide direct evidence of conditioned immunosuppression, per se (for example, depressed autoantibody titers). Nonetheless, the results are consistent with previous data (*2*) indicating that the pairing of saccharin and cyclophosphamide enables saccharin, acting as a CS, to suppress immunologic reactivity; they are also consistent with the hypothesis that such conditioning might thereby delay the onset of autoimmune disease under a regimen of chemotherapy that was not, in itself, sufficient to influence the development of SLE in comparison with an untreated control group. As such, these findings constitute an elaboration of the biologic impact of conditioned immunopharmacologic responses. The present study suggests, further, that there is some heuristic value in analyzing a pharmacotherapeutic regimen in terms of conditioning operations. Based on the present paradigm in which conditioned stimulus presentations (placebo treatments) were substituted for some active immunosuppressive therapy, it may be hypothesized that the prescription of a noncontinuous schedule of pharmacologic treatment in contrast to an analysis of the effects of continuous regimens of drug (or placebo) would be applicable in the pharmacotherapeutic control and regulation of a variety of physiologic systems.

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Endogenous Opiates and Energy Balance

Abstract. *Increasing the palatability of food has two opposite effects: it promotes overeating and provokes caloric output (energy expenditure). The increase in energy expenditure is too small to compensate for overeating and, as a result, obesity occurs. Repeated administration of zinc tannate of naloxone, a long-lasting opiate antagonist, completely abolishes this diet-induced obesity in rats. The drug accomplishes this not only by reducing overeating but also by increasing energy expenditure. This suggests that endogenous opioid peptides encourage obesity in two ways—by stimulating appetite for palatable foods and by reducing energy expenditures.*

In our Western societies the average adult gains about 13 kg between the ages of 20 and 50 years.

Certain animal species, such as rodents, follow a genetic program for an increase of adipose mass with age. This does not seem to be the human situation, since in non-Western societies average weight remains stable throughout adulthood and decreases slightly with old age. This has led to a general consensus that the weight gain observed in modern societies is essentially due to environmental conditions. The three main factors usually mentioned are the availability of a great variety of palatable foods, the decrease in energy expended, and the multiplication of stressors. That three factors usually occur together makes it difficult to appreciate their individual effects.

The only available experimental demonstration in human subjects concerns food choice. Replacing the usual varied meals by monotonous free feeding, in subjects whose physical activity and stressors are not modified decreases weight, which then stabilizes at a lower level (*1*).

Replacing a laboratory animal's usual monotonous laboratory food by a large choice of highly palatable items ("cafeteria" diet) induces overeating and an increase in weight (*2*, *3*). When cafeteria rats are given more exercise, their overweight is diminished, but not to the level of controls (*3*). Tail pinching, a stressor, provokes overeating, obesity, and an increase in plasma β-endorphin (*4*).

We hypothesized that the overavailability of various palatable foods acts, like a stressor, through the endogenous opiate system, which in turn modifies the two components of energy balance—intake and expenditure.

We have shown that short-term injections of opiate antagonists suppress hyperphagia both in genetically obese rodents (*5*) and in cafeteria rats (*6*). We now report that the repeated administration of a long-lasting opiate antagonist drug prevents obesity induced by the cafeteria diet by diminishing overeat-

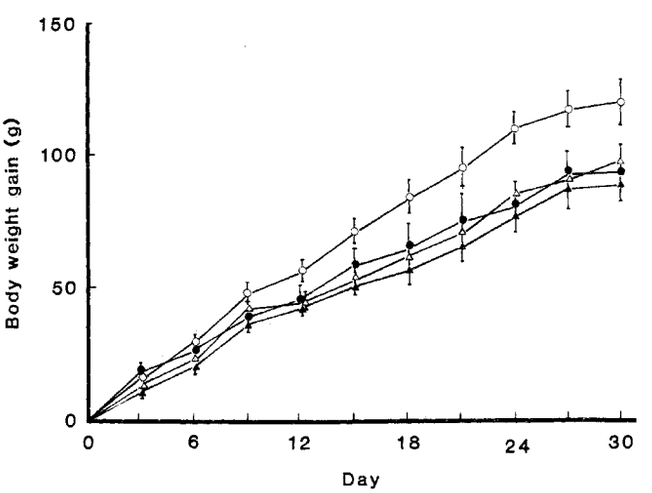

Fig. 1. Mean body weight gains. At day 30, the group given a cafeteria diet but no ZTN (○) had gained significantly more weight (analysis of variance, $P < .05$) than any of the other groups, none of which differed from each other. Symbols: ○, cafeteria-vehicle; ●, laboratory-food–vehicle; △, cafeteria-ZTN; and ▲, laboratory-food–ZTN.

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