Table 1. Changes in distribution of percentage of variability accounted for as a function of the number and nature of the predictors of consumption, without regard to food classes.

Predictor Satiety	Percentage of variance explained by						
	Individual predictors	Predictors in different combinations*					
	50	34		43	30	35	32
Preference	31		24	23	22	22	23
Percentage fat	31					17	12
Percentage protein	26						8
Log caloric density	43	24	37		21		
Total		58	61	66	73	74	75

\*  $\beta_{01.2...kr_{01}}$ ;  $\beta_{02.1...kr_{02}}$ , and so forth, where the  $\beta$ 's in the products (which are the percentage variances) in different columns are from separate regression equations.

expressed in percentages (for example, for fat, or for men taking a food) were converted to anglits to stabilize the variance. The data were then subjected to multiple linear regression analyses. The results are expressed as the percentage of variability accounted for, that is,  $R^2$ .

The  $R^2$ , for all predictor variables, is 0.77. However, because some of the variables are highly related to one another, some could be eliminated by a search for the minimum number of them that could explain most of the 77 percent of the variability. It was found that 75 percent could be accounted for by four variables: satiety, preference [from the national surveys, (1)], percentage fat, and percentage protein. Of these, satiety and preference are the most important in terms of their contribution to the prediction of consumption. They explain about 55 percent (Table 1, last column: 32 + 23percent) of the variability when in combination with the fat and protein values. When preference and satiety are taken alone as the only predictors, they account for 66 percent of the variation. The results for various combinations of the most important predictors are shown in Table 1.

Conversion of fat and protein content to percentage of total calories reduced their predictive abilities. This reduction is due to the fact that the conversion indirectly eliminates the role of water content; but percentage water, which has appreciable predictive power (29 percent; r = -.54), is, of course, inversely related to caloric density (r =-.95). Thus, protein and fat amounts per se are more important than their interrelationships with each other and with carbohydrate. Not surprisingly, the logarithm of caloric density may be substituted for amount of fat with almost equal effectiveness as a predictor.

If one is looking only for predictors, protein could be omitted since it raises the predictive value by only 1 percent (see the last two columns of Table 1). In fact, if empirical prediction were the problem, knowledge of the food classes alone is almost sufficient. The foods were divided into six classes: main dishes, starches, vegetables, salads, fruits and desserts, and breakfast items; then. knowing merely the class in which a food lies enables one to predict about 60 percent of the variation in percentage taking among all foods. A regression with adjustment for class differences (6), employing the four most important variables and the six food classes, yields an unbiased  $R^2$  of 0.82. However, these classes in themselves are reflections of more basic characteristics of the foods; hence they shed no light on the psychological and physiological or nutritional factors that regulate food acceptance in man.

Although many factors in eating behavior have been postulated or demonstrated, relatively few variables appear to guide normal adult human eating. Each of the four variables (last column, Table 1) contributes appreciably to the explained variance, and all appear to be basic factors (7).

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# Arousal Effects on Evoked Activity in a "Nonsensory" System

Abstract. Responses from motor cortex evoked by cerebellar stimulation have been recorded with chronically implanted electrodes from cats in different states of arousal. The response, which in the waking cat consists of a short latency biphasic component followed by one or more slower waves, was attenuated, or abolished completely, in association with electroencephalographic and behavioral signs of decreased arousal. In contrast, responses in primary somatosensory cortex evoked by stimulating the bulbar trigeminal nucleus were enhanced during periods of decreased alertness.

During the last several years, evidence for a concept of "central control of afferent activity" has been derived from experiments in which the configuration of evoked responses in sensory systems has been shown to be modifiable by various experimental interventions (1). These maneuvers have included: direct and indirect stimulation of the brainstem and thalamic reticular activating system; repeated presentation of stimuli to produce "habituation"; and association of positive and negative reinforcement with sensory stimuli.

There is no reason a priori to assume that similar plastic effects might not be demonstrable in "nonsensory" or "central-central" systems, that is, systems which are relatively remote from peripheral inflow to (or outflow from) the central nervous system and which are not ordinarily considered part of the classical sensory or "peripheral-central" input pathways. Indeed, some investigations already support this possibility (2). If this is the case, "central control of afferent activity" may be only part of a more general phenomenon in which activity of various systems, "peripheral-central" and "centralcentral" as well, is modified as the functional state of the animal changes. With this in mind we investigated the relations between state of arousal, defined by behavioral and electroencephalographic (EEG) criteria, and the configuration of evoked activity in a nonsensory, "central-central" system. A classical sensory system was also studied for comparison.

Cats were implanted under pentobarbital anesthesia with bipolar electrodes made of two strands of No. 36 gauge stainless-steel wire twisted together leaving 1 to 2 mm between the tips. In seven cats electrodes were placed, among other sites, on the motor cortex (anterior sigmoid gyrus, about 5 to 10 mm from the midline) and into the cerebellum in the vicinity of the nucleus interpositus, which has been reported to be the origin of the cerebellar outflow to motor cortex by way of the ventrolateral nucleus of the thalamus (3). These placements were adjusted during operation so that cerebellar stimulation would evoke a response from the motor cortex. The electrodes were led out to a Sheatz pedestal (4) which was mounted over the frontal sinus and which served as the ground.

During recording the cat was placed unrestrained in a chamber (191/2 by  $19\frac{1}{2}$  by 32 inches) with a Plexiglas front to permit observation of the animal's behavior. Square wave shocks, typically 8.0 volts lasting 0.05 msec (near maximal in most experiments), were delivered to the cerebellar electrode. Signals were picked up monoor bipolarly and were led from the cat with Microdot wire cable to conventional RC-coupled preamplifiers. The activity was displayed oscilloscopically and simultaneously on a conventional EEG. During most experiments a measure of bodily movement was also recorded. The bottom pan of the recording cage was mounted on stiff springs to form a system which could be set into slight oscillation by the cat's movements. These oscillations were picked up by ordinary phonograph cartridges and were amplified so that the minimal movements of an alert cat lying quietly could produce a detectable signal while rapid vigorous movements would overdrive the recording system.

Records were taken from cats over a wide range of levels of alertness. Sleep was ordinarily facilitated by placing the animal in a slowly rotating drum or in a shallow pan of water to keep it awake for periods up to 18 hours prior to recording sessions. Figure 1 reproduces records selected from two consecutive days of observation on a single cat. The left-hand portion shows ten superimposed oscilloscopic traces of the motor cortex response evoked by stimulating in the vicinity of nucleus interpositus in the cerebellum; the right-hand portion illustrates the background EEG during which the responses were recorded. When the cat is awake, sitting or lying quietly, the evoked response consists of a short 8 FEBRUARY 1963

latency biphasic response usually followed by one or more slower waves (Fig. 1, C and D). The earliest sign of evoked activity has a latency of about 2 msec and the major events of the response are over in about 100 msec or less.

When the cat moves about and the EEG is nearly maximally desynchronized, the amplitude of the evoked response is attenuated (Fig. 1, A and B),

weakly and inconstantly in the initial wave but sometimes quite markedly in the later components. The latter also often show a marked reduction in latency and duration. As the cat becomes drowsy and the EEG shows increasing synchrony, profound changes occur in the configuration of the evoked response (Fig. 1, E-H). The later components first increase in latency and duration and decrease in amplitude un-

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Fig. 1. (Left) Ten superimposed responses from motor cortex (anterior sigmoid gyrus) evoked by cerebellar stimulation; calibration: 100  $\mu$ v, 10 msec. (Right) EEG and movement records showing background during which evoked responses were recorded; AS, anterior sigmoid gyrus; ASS, anterior suprasylvian gyrus; calibration: 100  $\mu$ v, 1 sec.

til they disappear altogether, leaving only the first deflection, the amplitude of which fluctuates markedly. This component also often disappears entirely for extended periods when the EEG shows the large slow waves characteristic of deeper sleep.

These data demonstrate that major changes occur in the evoked responses generated by activating a nonsensory, "central-central" system, and that these changes apparently correlate with changes in the background EEG and associated states of arousal. That the strength of stimulation was near maximal serves to emphasize the power of this central control phenomenon.

In contrast to the present results, most investigators report that under their experimental conditions evoked responses in sensory systems usually become larger in amplitude and duration in association with behavioral and EEG signs of reduced arousal. We have been able to reproduce this typical result under our experimental conditions in the primary cortical projection of the bulbar trigeminal nuclear complex (5). In this system the cortical response evoked by stimulating various points in the fifth nerve nucleus is enhanced in association with increased EEG synchrony and is attenuated when the background EEG is desynchronized. We have also demonstrated these opposite modifications of the cerebellocerebral and trigemino-cerebral projections taking place in the same cat simultaneously.

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## **Reversible Agglomeration Used To Remove Dimethylsulfoxide from** Large Volumes of Frozen Blood

Abstract. The clumping that takes place when blood is mixed with isotonic glucose is well known to the clinician. This curious phenomenon has been characterized and used to prepare blood frozen in the presence of dimethylsulfoxide for transfusion. A hypothesis is advanced to explain the mechanism of reversible agglomeration of erythrocrytes in nonelectrolyte solutions

Every surgeon has witnessed the clumping of erythrocytes that takes place within the intravenous set when a blood transfusion is followed by isotonic glucose in water. This commonly observed phenomenon is worrisome, but does no apparent harm to the patient.

In experiments undertaken to find a simple method for removing the preservative agent dimethylsulfoxide from thawed blood, we suspended 1 volume of packed human red cells in 10 volumes of isotonic sucrose. Within 3 minutes, progressively larger aggregates of cells formed, which settled to the bottom of the container. More amazing was the further discovery that resuspension of the agglomerated cells

occurred after the addition of an equal volume of plasma.

Reversible agglomeration of blood cells was further studied to characterize the phenomenon. Aggregates were found to form in solutions of glucose or glycine as well as in sucrose. These diluents must range in concentration from slightly hypotonic (200 mosm/kg) to two and one-half times normal osmotic strength. At 5°C agglomeration takes place more slowly than at 20°C and occurs most rapidly at 40°C. The pH optimum for settling lies between 5.2 and 6.1. Between pH 6.1 and 6.5 clumping occurs, but settling is retarded. Above pH 6.5 no clumps form, and those previously formed at a lower pH resuspend. Dispersion also occurs and agglomeration is blocked by the presence of sodium chloride in concentrations greater than 0.02M.

These parameters, plus the known tendency of y-globulins to form reversible complexes with plasma  $\beta$ -lipoproteins in the pH range 5.2 to 6.1 (1), permitted a tentative hypothesis to be advanced. At a pH between 5.2 and 6.1  $\gamma$ -globulins in the plasma might form a reversible complex with the lipoprotein of the walls of red blood cells. Reduction of the ionic strength of the medium by dilution with a nonelectrolyte might then precipitate the  $\gamma$ -globulins with coprecipitation of the



Fig. 1. The sequence of events which occur after mixing 10 ml of packed human blood with 90 ml of 10-percent glucose: A, immediately after mixing; B, 30 seconds; C, 80 seconds; D, 120 seconds (the fuzziness is due to motion of the clumps not stopped by exposure time of 1/25 second); E, 5 minutes. Note agglomeration to less than the initial volume of packed cells.