References and Notes

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Operant Behavior during Sleep

Abstract. Operant-conditioning techniques were employed to produce organized behavior during sleep as defined by the electroencephalographic record. Patterned responding was found in so-called "light" and "deeper" electroencephalograph stages as well as in the awake record. Results suggest that the use of the electroencephalographic record as a reliable indicator of the sleep-awakefulness continuum be re-examined.

The numerous investigations of the role of the reticular formation (*1*), and single-unit responses (*2*), in the modification of on-going behavior have led to several speculations about how the central nervous system subserves sleep. Of equal interest has been the problem that operative functions normally thought to be characteristic of waking behavior alone are present also in the sleep state—functions such as discriminative responses (*3*), integrative behavior, and control of on-going activity. The problem appears to be in the inadequacy of traditional behavioral and physiological indices to define a state of sleep. This report shows that complex behavior can be produced while physiological indicators, such as the electroencephalographic record, signal what has been defined as "deep sleep."

The experiments were done on five subjects ranging in age from 17 through 28 years. The subjects were tested weekly. The normal routine was for subjects to report to the laboratory on the night preceding the testing night. Suitable activities were provided during the intervening period, and the testing session was begun as closely as possible to each subject's normal bedtime hour. All subjects were thus deprived of sleep for about 36 to 40 hours.

Operant-conditioning techniques (*4*) were employed in the following manner. A microswitch was taped to each of the subject's hands, and two electrodes for administering shock were attached to the lower right leg. Whenever 3 seconds elapsed without a response on the left-hand microswitch, a pulse was delivered to the leg. By responding at a rate greater than once every 3 seconds the subject could avoid all shocks. A schedule of "time-out" periods, each of which followed a fixed number of responses (fixed ratio), was programmed on the right-hand key. During a time-out period, lasting either 5 or 8 minutes, all electrical equipment was turned off and the subject was allowed to sleep. The time during which avoidance and ratio-key responding were programmed constituted the scheduled activity period. Ten seconds before the end of a time-out period, a loud buzzer was sounded next to the subject's ear. The buzzer sounded continuously for 10 seconds and terminated with the appearance of the signal light mounted in front of the subject's head, initiating the next scheduled activity period. The first shock was delivered 3 seconds after the onset of the signal light unless the avoidance key was operated. This cycle of events was repeated continuously throughout the session.

Continuous electroencephalographic records were made from several scalp placements. The bulk of the data reported here was taken from bipolar placements of electrodes on the occipital center line. Parietal and frontal monopolar recordings were also obtained and were used in substantiating the various stages of sleep.

The scoring of the sleep stages followed a classification suggested by Dement and Kleitman (*5*) with some modifications. Stage 1A was taken as that portion of the record still exhibiting alpha bursts; stage 1B covered that part of the record between the last alpha burst and the appearance of the first 14-cy/sec spindle and consisted mainly of 3- to 6-cy/sec activity; stage 2 was characterized by spindle activity with low-voltage background, including some 3- to 6-cy/sec activity together with biparietal humps and K-complexes;

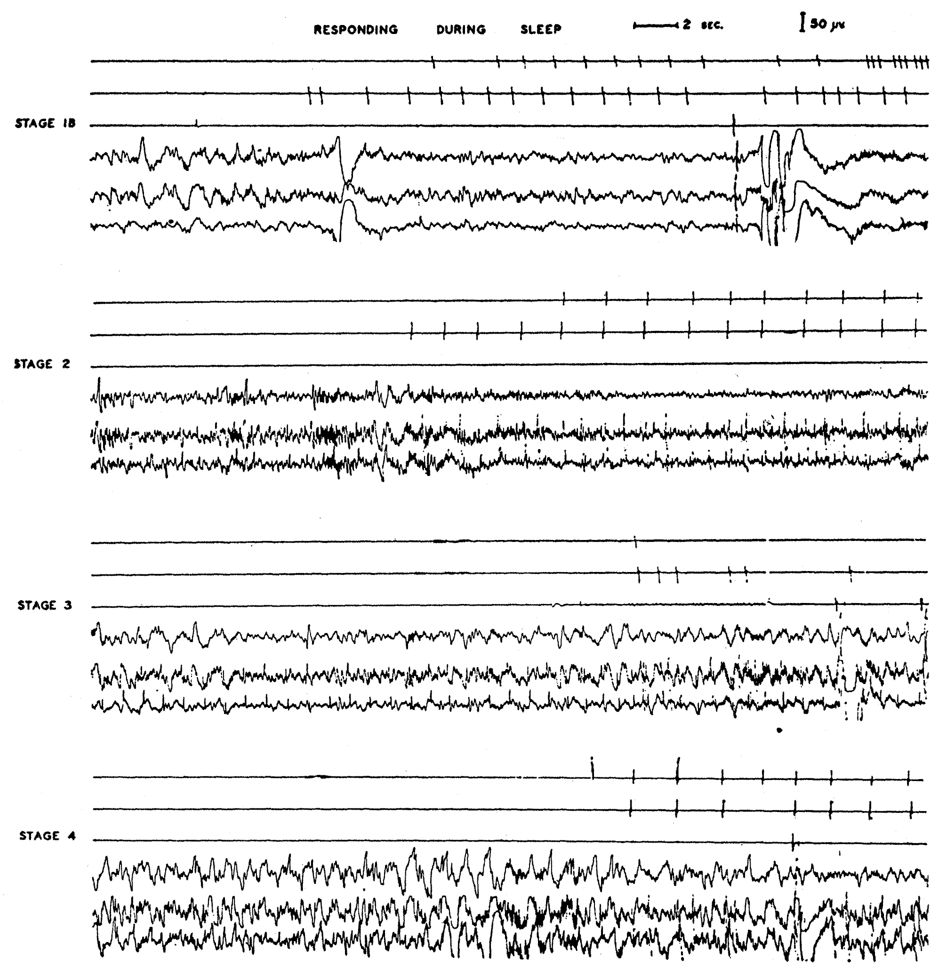

Fig. 1. Responding during electroencephalograph sleep. Stages are defined in the text. The six pens for each stage show from top to bottom: (i) fixed-ratio reinforcement responses, (ii) shock-avoidance responses, (iii) shock administration, (iv) occipital bipolar placement, (v) and (vi) occipital monopolar placements to the left-ear reference for monitoring. The examples of stages are taken from separate subjects.

stage 3 was signaled by the appearance of high-voltage slow waves (greater than 100 μv) with some 14-cy/sec spindling; stage 4 covered that part of the record where waves larger than 100 μv in the 1- to 2-cy/sec range occurred at a rate greater than two per 10-second sample of record.

The results showed in general that all subjects displayed an electroencephalograph sleep record and learned to respond appropriately on both microswitches without returning to the electroencephalograph waking state. Moreover, the data indicated that patterned activity could occur when the subject was in any electroencephalograph sleep stage, including stages 2, 3, and 4, although most of the evidence was accumulated from stage 1B. Figure 1 shows responding during the four stages of sleep as defined above.

Of particular interest is the occurrence of responding during the time-out periods. Such responding occurred in sequences lasting from 2 to 20 seconds and resembled the performance of the subject during normal activity periods in rate and pattern. All subjects exhibited this behavior, although its occurrence was relatively infrequent and was seen only in the early sessions. The corresponding electroencephalograph stage was classified as 1B or 2 in all cases but one. Behavior of this type may have occurred because the experimental conditions appeared ambiguous to the subjects in the beginning trials. In keeping with Jasper's notion, it may very well be that the subject must learn not to respond to "irrelevant stimuli" (*1*, p. 320).

These observations of organized behavior during sleep, together with studies involving discriminative responses previously reported, lend support to the idea that the "higher mental states" continue to operate at some level of the nervous system even during *deep* sleep. Perhaps such complex behavior does not require an alert and active brain as defined by the electroencephalograph record, but perhaps it may be that our means of assessing levels of sleep and concomitant activity need to be revaluated.

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Control of Osmotic Pressure of Culture Solutions with Polyethylene Glycol

Abstract. Experiments with kidney beans indicate that Carbowax polyethylene glycol, molecular weight 20,000, upon purification, may be used as an agent to control the osmotic pressure of plant nutrient solutions without the hazard of interference with normal metabolic processes. With the sodium electrode and the thermocouple psychrometer, interaction between ions and Carbowax is shown to lead to a slight dissociation of the latter.

In the course of studies dealing with the aqueous environment of plant and animal organisms, it has become necessary to discern between effects due to the total level of solute (osmotic effects), and those due to the ionic composition at any such level (ionic composition effects). Only under conditions where an osmotic agent which does not interfere with normal metabolic processes is involved is it possible to separate osmotic effects from ionic composition effects.

In the present study, 13 different compounds were selected for examination on the basis of their chemical structure. A majority consisted of polymers used in the plastics industry. As a first step, the compounds were screened according to solubility or miscibility, viscosity, and resistance to chemical as well as biological activity. Subsequently, the compounds that passed these tests—dimethylsilicone (*1*), Elvanol 51-05 polyvinyl alcohol (*2*), polyvinylpyrrolidone NP-K30 (*3*), and Carbowax polyethylene glycols with molecular weights of about 4000, 6000, and 20,000 (*1*)—were investigated with regard to their effect on plant growth. Solution culture studies involving dwarf red kidney beans (*Phaseolus vulgaris* L.) indicated that all of the compounds were toxic when applied in their commercial form in concentrations equivalent to 1 atm of osmotic pressure.

The order in which the aforementioned compounds are listed is one of decreasing toxicity. In the case of Carbowax of molecular weight 20,000, spectrographic analysis (*4*) indicated the presence of large amounts of aluminum and magnesium. Purification of the polyvinylpyrrolidone and the two polyethylene glycols of highest molecu-

Table 1. Total fresh weight per plant of red kidney beans (average of four plants) grown in nutrient solutions containing either an excess of ions (in excess were the ions Na^+, Mg^{++}, and Cl^-; Ca^{++} and Mg^{++} were present in a ratio of 3:1, and the value of the ratio of Na^+ to $(Ca^{++} + Mg^{++})^{1/2}$ was 3.1 $mmole^{1/2}$ $liter^{-1/2}$ for all solutions) or one of three osmotic agents of high molecular weight [carbowax of molecular weight 20,000 (C20M) and 6000 (C6M), and polyvinylprrolidone (PVP), all purified by dialysis].

Osmotic pressure (atm)		Fresh wt (g per plant)			
		Osmotic agents			No osmotic agents
Ions	Osmotic agents	C20M	C6M	PVP	
		With osmotic agents			
1.4	0.5	86	54	62	
1.4	0.9	77	32	17	
2.5	0.5	64	43	42	
		With excess of ions			
1.9					83
2.3					72
3.0					63

Table 2. Properties of ionic solutions* of Carbowax polyethylene glycol of molecular weight 20,000 (C20M). Ca^{++} and Mg^{++} were present in proportions of 3:1, and the value of the ratio of Na^+ to $(Ca^{++} + Mg^{++})^{1/2}$ was 3.1 $mmole^{1/2}$ $liter^{-1/2}$ for all solutions.

Osmotic pressure (atm)		Mean activity coefficient of NaCl†	Osmotic pressure (% difference)‡	Relative viscosity (seconds of outflow)§	r_i
Ions	C20M				
1		0.92		9.5	
	1	1.03	+0	21.8	82
	2	1.08	+3	47.0	109
	3	1.25	+3	86.1	134
2		0.89		9.2	
	1	0.91	+2	21.0	82
	2	1.04	+4	46.4	111
	3	1.08	+3	82.2	134
3		0.87		9.4	
	1	0.94	+10	20.5	81
	2	1.08	+14	45.7	108
	3	1.11	+11	81.9	134

* The cations present were Na^+, Ca^{++}, and Mg^{++}; the anion was Cl^-. One atmosphere of osmotic pressure corresponds to solutions of about 0.024*N* NaCl or 0.032*N* $CaCl_2$. † As measured with the Beckman No. 78178V sodium selective electrode. ‡ The deviation of the osmotic pressure of mixed solutions of ions and C20M from the sum of the osmotic pressures of separate solutions of ions and C20M, as registered by the thermocouple psychrometer. § Of a 10-ml aliquot from 10-ml pipette.