Dialysis of Sleep and Waking Factors in Blood of the Rabbit

Abstract. *Dialysis of cerebral venous blood was performed on rabbits during sleep induced by electrical stimulation of the hypnogenic thalamic system or during sustained wakefulness elicited by stimulation of the activating reticular system. The injection of dialysate from a sleeping donor elicited in a normal recipient a moderate sleep which did not differ from normal sleep according to the behavior of the animal and the pattern of the electroencephalogram. The action of the dialysate obtained from sleeping donors was significantly different from that obtained from control donors after sham-stimulation of the thalamus. Injection of dialysate from alert donors elicited a behavioral and electrographic arousal with rhinodiencephalic activation symptoms. These results suggest that dialyzable humoral factors play a role in the mediation of sleep and wakefulness.*

Various experimental data suggest that sleep and arousal can be transmitted humorally from a donor to a recipient animal. Thus, the injection of cerebrospinal fluid from experimentally fatigued dogs into the fourth ventricle of normal dogs elicits a sleep-like state in the latter, attributed to a hypnotoxin (*1*, *2*). In cross-circulation experiments, sleep elicited in the donor by electrical stimulation of the intralaminary thalamus induces, after a short latency, a similar sleep in the recipient [Kornmüller *et al.* (*3*) in the cat; Monnier *et al.* (*4*) in the rabbit]. Similarly, the alert state elicited in the donor by electrical stimulation of the midbrain reticular formation induces arousal in the recipient [Purpura (*5*) in the cat; Monnier *et al.* (*4*) in the rabbit].

We have now conducted experiments to determine whether psychotropic factors are present in the donor's blood during experimental sleep or alertness, and whether such factors can be extracted by hemodialysis. To test the action of the fresh dialysate obtained from sleeping, waking, and control donors, we injected small amounts (20 ml) of dialysate into free-moving recipients.

By the method of Koller *et al.* (*6*), venous blood was made to flow through a metal canula inserted in the confluens sinuum; the blood was further propelled by means of a roller pump through a dialyzer and made to flow back through a polyvinyl catheter to the femoral vein (Fig. 1). As the dialyzer, we used the artificial kidney of Kuhn *et al.* (*7*), which works with a very small blood capacity (5 ml) and a small amount (30 ml) of dialyzing fluid (*8*). (If psychotropic humors were dialyzable, they would appear in greater concentration in a small amount of dialyzing fluid.)

During dialysis, electrical activities of brain, heart, and respiration were recorded continuously. We analyzed the EEG's by means of an automatic frequency analyzer (*9*) and performed a statistical comparison of the various data before and during stimulation. Similarly, we compared the data from sleeping and waking donors with those of control donors (*t*-test). As a quantitative criterion of variations in wakefulness, we adopted the sum of the delta activities, given on one channel of the automatic frequency analyzer, the spindle activities, the duration of desynchronized cortical activities in seconds, and finally the theta activity of the hippocampus, summed on the second channel of the frequency analyzer. Sleep was expressed by increased delta and spindle activities; alertness was expressed by increased desynchronization episodes in the motor cortex and theta rhythms in the hippocampus (Fig. 2).

We induced sleep in the donors by repetitive electrical stimulation of the medio-central intralaminary thalamus

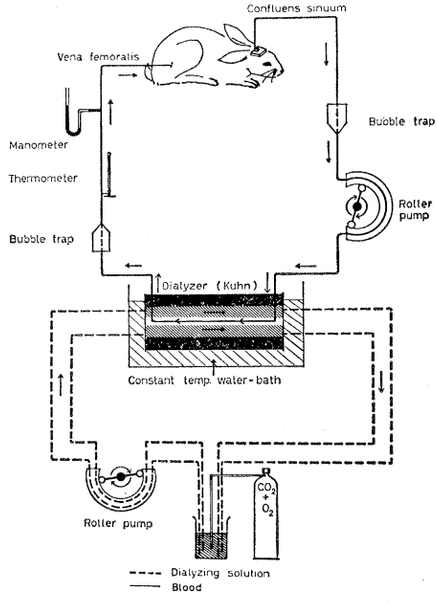

Fig. 1. Schematic representation of the dialyzing system, including the hemodialyzer of Kuhn *et al.* (*7*).

(frequency, 6/sec; voltage, 0.7; pulse duration, 12 msec; duration of each stimulation, 30 seconds) (*10*). Arousal was induced by repeated stimulation of the midbrain reticular formation (frequency, 150/sec; voltage, 0.5 to 0.7; pulse duration, 0.5 msec; duration of stimulation, 15 to 20 seconds). Control experiments were performed under similar conditions, but only with sham-stimulation of the thalamus or reticular system (that is, with electrodes in the brain but without stimulating current). The *p*H and the concentration of the chief electrolytes (Na^+, K^+, Ca^{++}, inorganic phosphorus) were determined in the blood and the dialysate at the beginning and toward the end of the dialysis.

In each experiment we first performed a preliminary dialysis which lasted 40 minutes; the real dialysis was then performed for 80 minutes, with simultaneous stimulation of the mentioned brain centers. After the dialysis, we injected 20 ml of the dialysate into the auricular vein of a free-moving recipient. The electrical brain activity of this animal was recorded for 20 minutes before and 40 minutes after the injection. The motor behavior was filmed and registered with a kinesimeter. Here again, we compared statistically (*t*-test) the values before and after the injection, and the values of control animals which received dialysate from sham-stimulated donors. Values of *p* greater than 5 percent were considered as not significant.

Our experiments were performed on 55 rabbits (10 sleeping donor-recipient pairs; 11 waking donor-recipient pairs; 5 control donor-recipient pairs, and 3 control animals with different *p*H).

Stimulation of the hypnogenic intralaminary thalamus in the donor induced sleep with increased delta activities and spindles arising from the cortex. In control donors (during sham-stimulation of the thalamus), there was a tendency to increased wakefulness with decreased delta activity and increased desynchronization episodes. The continuously recorded heart and respiration rates, the temperature (rectal), the *p*H, and the concentration of definite electrolytes in blood and dialysate did not show significant differences between sleeping donors and control donors.

Stimulation of the midbrain reticular system (150 cycles per second) induced in the donor an EEG typical of arousal with desynchronization of corti-

cal activities and theta rhythms in the hippocampus. The statistical analysis showed that there was a significant tendency to alertness as compared with the preliminary basic values.

After intravenous injection of 20 ml of the dialysate, the following observations were made.

The dialysate from control donors (after sham-stimulation of the thalamus) slightly activated the behavior and the EEG's of normal recipients (Fig. 3*A*). The delta and spindle activities decreased, but not very significantly, compared with the values before the injection.

The dialysate from a sleeping donor induced in the recipient a sleep of moderate intensity which did not differ from physiological sleep as shown by the EEG and by the behavior of the animal (Fig. 2*A*). This sleep could be reversed immediately by tactile or acoustic stimuli, as in normal sleep. The quantitative analysis showed that there was a marked increase of slow delta activities in the motor cortex 10 to 15 minutes after the injection (Fig. 3*A*), with a maximum occurring between 25 and 40 minutes. This increase is statistically significant when compared to the values before the injection (in the same animal), and when compared with the control animals (dots).

The dialysate from an alert donor, activated by stimulation of the midbrain reticular formation, induced in the recipient an alert behavior, usually with several rhino-diencephalic symptoms such as restlessness, tendency to escape, licking of genital organs and coprophagy, similar to those elicited by electrical stimulation of the hippocampus and related hypothalamus (*11*). As shown on the EEG's, desynchronization episodes in the neocortex and theta activities in the hippocampus increased (Fig. 2*B*). The quantitative analysis showed a significant decrease of delta activities in the cortex (Fig. 3*B*) and increased theta activities in the hippocampus, as compared to values before the injection. However, the delta decrease was not significant when compared to the values of the control animals, but was very significant relative to the effect of dialysate from a sleeping donor. The dialysate from a waking donor seemed to have a faster action than that from a sleeping donor.

The fact that sleep and alertness may be transmitted humorally (by the hemodialysate) is in agreement with previous observations. For instance, we have already mentioned that intracisternal injection of cerebrospinal fluid of a fatigued dog into a normal dog induces sleep in latter (*1*). Schnedorf and Ivy (*2*) confirmed this sleep-like depression of the central nervous system. Similarly, in animals with cross circulation, stimulation of the intralaminary thalamus induces electrographical sleep both in donor and recipient [Kornmüller *et al.* (*3*) in the cat; Monnier *et al.* (*4*) in the rabbit]. On the other hand, Purpura (*5*) demonstrated in cats with "encéphale isolé" and cross-circulation that stimulation of the bulbar reticular formation elicits an electrocortical activation in the recipient, suggesting a humoral transmission.

Our experiments support the conception that sleep and alertness can be transmitted humorally, since dialysate from sleeping or alert donors induce corresponding alterations of wakefulness in the recipients. It is not yet possible to specify the nature and origin of the dialyzed psychotropic humors, but control experiments already allow us to state that the observed effects are not due to such factors as circulation, respiration, *p*H, or the electrolytes Na, K, Ca, and inorganic phosphates.

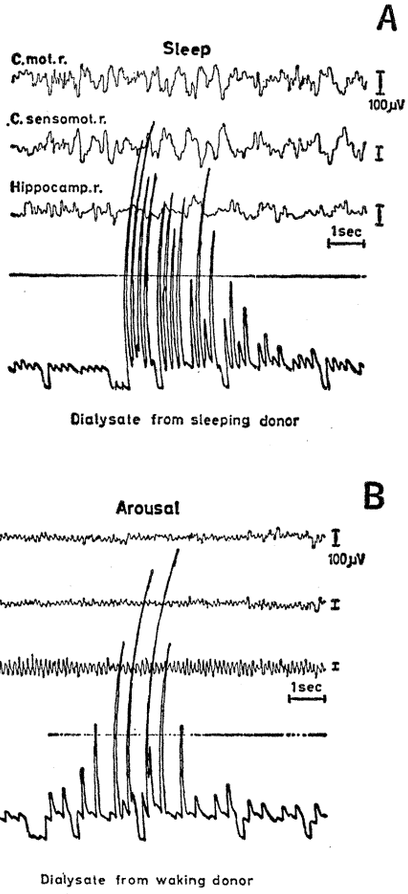

Fig. 2. Electroencephalograms showing (*A*) sleep induced in normal recipient (rabbit) by injection of 20 ml of dialysate from a sleeping donor (slow delta activities in the cortex are indicated by increased deflections of the frequency analyzer in the delta range); and (*B*) arousal after injection of dialysate from waking donor (desynchronized cortical activities with enhanced theta rhythms are shown in the hippocampus).

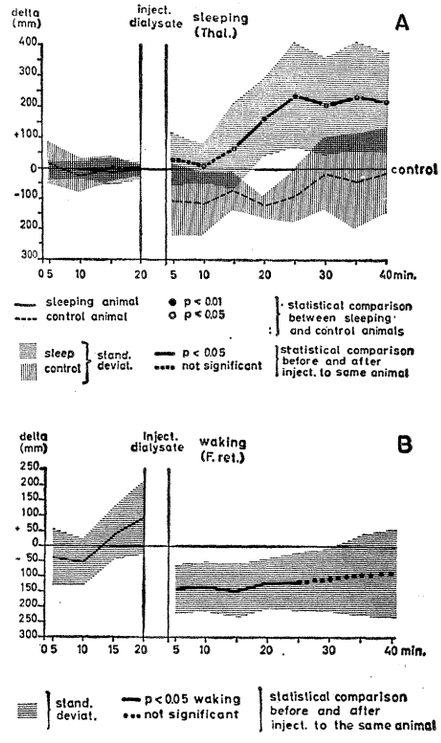

Fig. 3. *A*, Sleep with significant increase of delta activities in recipients after injection of 20 ml of dialysate from sleeping donors (10 animals). Recipients receiving control donor dialysate show tendency to wakefulness, but the result not statistically significant when compared with the values before the injection (10 animals). Before the injection there was no significant delta difference in both groups. *B*, Alertness with significant delta decrease in recipients after injection of 20 ml of dialysate from alert donors (compared with the values before injection; 11 animals).

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8. The dialyzing fluid had the following composition: NaCl, 6.6 g; $NaHCO_3$, 2.25 g; KCl, 0.3 g; $MgCl_2 \cdot 6 H_2O$, 0.1 g; $CaCl_2 \cdot 2 H_2O$, 0.2 g; glucose, 2.0 g/liter.
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Attitude Statements as Positive and Negative Reinforcements

Abstract. *The finding that attraction is a function of attitude similarity has been interpreted as a special case of the effect of positive and negative reinforcements on attraction. A simple discrimination learning task was employed in which the reinforcements were attitude statements similar and dissimilar to the opinions of the subject. The presentation of similar attitude statements after each correct response and dissimilar attitude statements after each incorrect response significantly changed response probability. The hypothesis that such statements could be used as reinforcers in a learning situation was thus confirmed.*

Attitude is a construct which refers to an enduring, learned readiness to behave in a consistent way along an affective dimension toward a given object or class of objects. On the basis of a variety of antecedent experiences, each individual holds a large number of beliefs, opinions, values, and judgments which involve attitudinal components. The relative similarity or dissimilarity of the attitudes of any two individuals has been found to exert a significant effect on their mutual attraction.

In the typical experimental study of attitude-similarity and attraction, the investigator obtains a sample of the attitudes of a group of subjects, later presents the subjects with the real or purported attitudes of a stranger, and measures the subject's attraction toward this stranger. It is a consistent finding that attraction toward the stranger is a positive linear function of the proportion of that stranger's attitudes similar to those of the subject (*1*).

In accounting for this relationship, one approach has been to interpret the effects of attitude similarity-dissimilarity as a special case of the effect of positive and negative reinforcements on attraction (*2*). Briefly, the rationale is that there is a learned drive to be logical and to interpret the environment correctly. With respect to the physical world, objective criteria are available to denote correct and incorrect behavior. Positive and negative reinforcements are usually provided immediately in terms of direct perceptual feedback (for example, fire is hot, stones are hard). There also exists, however, a complex social environment in which there are equal demands to be logical and correct but for which the only criterion is that of consensual validation. Thus, agreement by others concerning political affiliations or religious practices or morality acts as a reward in that it provides evidence that one is functioning in a logical and correct manner. Consensual invalidation and hence punishment occurs when there is disagreement by others. To date, there has been no direct test of the proposition that similar and dissimilar attitude statements act as reinforcing stimuli.

In the investigation described here, a simple discrimination learning task was employed in which the traditional reinforcements were replaced by statements of attitudes. If the reinforcement interpretation of attitude similarity-dissimilarity is correct, such statements should act to change behavior. Specifically, it was hypothesized that the probability of the occurrence of a response increases if that response is followed by the presentation of a statement consonant with an attitude held by the responder and decreases if that response is followed by the presentation of a statement dissonant with an attitude held by the responder.

A 45-item attitude scale was administered to over 100 students enrolled in introductory psychology at the University of Texas. The items concerned such topics as birth control, political parties, and belief in God. Subjects responded to each item on a six-point scale. For the experiment itself, 60 subjects who had relatively extreme views (1, 2, 5, or 6 on the scale) on at least 20 topics were selected.

The learning task consisted of a simple discrimination problem. The subjects sat in front of a large wooden apparatus which contained a window for the presentation of the stimulus

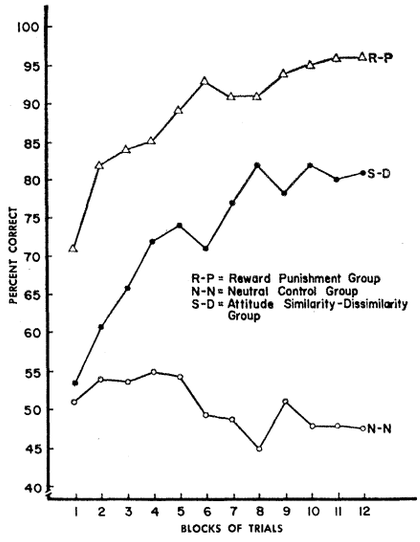

Fig. 1. Learning curves for the three groups of subjects showing percentage of correct responses over eight-trial blocks as a function of experimental conditions.

cards. A total of 96 cards was used, one for each trial, each containing a circle and a square of which one was black and one white, one large and one small. Each of the eight possible combinations of shape, size, color, and position appeared in random order in each block of eight trials. All subjects, run individually, were told that the experiment dealt with learning. When a stimulus card appeared in the window, the subject chose one of the two figures and said it aloud. Immediately afterward, a card was presented through a slit. The subject read the information printed on the card and then disposed of it in a discard box.

The 60 subjects were randomly assigned to one of three experimental conditions. The discrimination to be learned was small-large. In each group, small was correct and large incorrect for half of the subjects with the reverse for the other half. In the traditional reward-punishment group, the choice of the correct stimulus was followed by a card saying "RIGHT"; choice of the incorrect stimulus was followed by "WRONG." The attitude similarity-dissimilarity group received cards containing statements agreeing or disagreeing with their own position on one of the 20 topics about which they had strong views, depending on whether they gave correct or incorrect responses. Examples are: "There is definitely a God," or "There is no God"; "The Democratic Party is best," or "The Republican Party is best." Re-