mals, then it would be expected that in some hamsters stimulation from the olfactory system alone, after vomeronasal deafferentation, would be insufficient to achieve threshold levels. However, the converse would not be expected, because relatively less interference with the total system is effected by olfactory deafferentation only.

The results of our previous experiments with zinc sulfate suggested that the loss of mating behavior following bilateral bulbectomy was not due to peripheral anosmia (*4*). It is now apparent that peripheral deafferentation can mimic the effects of bulbectomy on male hamster sexual behavior if both the olfactory and vomeronasal systems are rendered nonfunctional. Bilateral bulbectomy produces this effect because both the main and accessory olfactory bulbs are typically removed. Even if the accessory bulb is spared, the vomeronasal nerves are inevitably damaged in the process of removing the main olfactory bulbs. Our results emphasize the necessity for determining to what extent both the primary olfactory and the vomeronasal systems are impaired by techniques designed to induce peripheral anosmia.

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12. One-half milliliter of zinc sulfate (5.0 percent in 0.5 percent saline) was passed through the nasal cavities of etherized hamsters with a bent section of 18-gauge stainless steel tubing attached to a syringe. The fluid was introduced through the nasopharyngeal meatus and aspirated as it exited at the external nares. In some animals saline solution (0.5 ml of 0.5 percent) was administered identically. This was done as a control procedure preceding later behavioral tests, but histological examination of the olfactory and vomeronasal epithelia was not performed.
13. See Powers and Winans (*4*). Mating behavior tests lasted 10 minutes or until two ejaculations had occurred with an ovariectomized female. All mounts, intromissions, and ejaculations were scored on an event recorder. Females had subcutaneous Silastic implants of estradiol and received injections of 500 μg of progesterone 3 to 5 hours prior to use; these females were used only if they showed intense lordosis when placed with stud males.
14. Animals were anesthetized with Nembutal (70 mg/kg); the frontal bone over the anterior portion of the main olfactory bulbs was removed and the large venous sinus in the midline between the two bulbs was visualized. McClure UltraMicro scissors were positioned over the sinus and lowered 3 to 4 mm while open. After they were closed they were withdrawn, and the bleeding was controlled with pressure and Gelfoam. Animals receiving the sham-1 procedure were treated identically except that the scissors were lowered only 1 mm so that the sinus was cut but the vomeronasal nerves, lying below this level, remained intact. For sham-2 treatment, a 22-gauge hypodermic needle was oriented vertically and lowered 3 to 4 mm into the main olfactory bulb just lateral to the midline sinus on both sides. This was designed to produce olfactory bulb damage comparable to that associated with the nerve-cutting procedure, but without damaging the vomeronasal nerves.
15. Hamsters were perfused with 0.8 percent saline followed by 10 percent formalin in 0.8 percent saline. The rostral portion of the skull, from bregma to the external nares, with the brain in situ, was hardened in formalin, decalcified in 25 percent formic acid, washed, and embedded in a mixture of egg yolk and gelatin. Horizontal sections (40 μm) were cut on a freezing microtome and stained with cresyl violet.
16. We thank E. Valenstein for valuable criticism of the experiments and the manuscript. We thank P. Britten for histological assistance and D. Fleming for conducting the behavioral tests. Sponsored by NIMH research grant 2R01MH20811-03 to E. Valenstein.

7 August 1974; revised 18 October 1974

Changes in Protein Levels in Perfusates of Freely Moving Cats: Relation to Behavioral State

Abstract. *Perfusates from the brains of freely moving cats, obtained by means of a push-pull cannula, contain high concentrations of proteins. The levels vary in a cyclic fashion and are higher during rapid eye movement sleep than during the waking state. The proteins represent a distinctive class of tissue protein and their changing levels appear to reflect an alteration in the protein content of the extracellular space of brain related to behavioral state.*

Sleep can be induced by perfusing a cat's brain with a perfusate obtained from a sleeping cat by means of a push-pull cannula (*1*). Substances that influence temperature control and food ingestion are found in brain perfusates obtained with similar procedures (*2*). These results suggest that samples of the extracellular medium can be obtained in perfusion experiments with push-pull cannulas and that the perfusates contain regulatory substances.

In the experiments reported here, we analyzed the protein composition of perfusates obtained from the brains of cats during sleep and waking states. We examined proteins because of evidence that a polypeptide may be a sleep-inducing factor (*3*) and because proteins can serve in a variety of situations as regulatory substances. In a more general context, proteins and polypeptides are secretory products of a variety of cell types, including neurons in cultures (*4*) and in the peripheral nervous system (*5*), and it is of fundamental importance to determine whether proteins are present in substantial quantities and whether they can be collected from the extracellular space of brain.

Eighteen cats of either sex (2.5 to 3.5 kg) were implanted with a 17-gauge stainless steel tube that served as a guide for a cannula. Detailed procedures have been described (*1*). The guide tube was aimed at the mesencephalic reticular formation (MRF) but the tip terminated 7 mm above the MRF. Electrodes were implanted in the sensorimotor cortex, around the orbit of the eyes, and in the neck muscles for electroencephalographic, eye movement, and electromyographic recordings, respectively.

The perfusion experiments were started at least 1 week after surgery. At approximately 11:00 a.m., the cat was placed in a large observation chamber in a quiet laboratory room. A two-barrel (20- and 27-gauge) push-pull cannula was introduced into the guide tube and lowered into the MRF and hippocampus. The cannula was connected via polyethylene tubing to syringes driven by a Harvard infusion-withdrawal pump. The brain was perfused continuously with a sterile commercial Ringer solution at 1 ml/hour for 12 to 21 hours. Samples of the perfusate were removed from the tubing each hour by means of a three-way

valve attached to the syringes. When the experiments were completed, the brain of each cat was perfused in situ with 10 percent formalin and the location of the cannula track was determined by analyzing 50-μm frozen sections stained with cresyl violet. The protein content of the perfusate was analyzed by the Lowry method as modified for dilute solutions (*6*) with crystalline bovine serum as standard.

We found that MRF perfusates contained proteins at concentrations ranging from 23 to 206 μg/ml. Approximately 2.0 mg of protein per animal was collected over a 24-hour period. The concentration varied in a somewhat cyclic fashion (Fig. 1). Although the cyclic patterns differed for different cats as well as for the same cat perfused on two occasions, cyclic variations in protein content were found in the perfusates of every cat. The protein concentration in perfusates of the hippocampus also showed a cyclic appearance. However, the variations did not directly relate to those observed for the MRF.

Several findings suggest that the cyclic changes in protein content of the perfusates were due to changes in the extracellular environment of the brain region perfused. The effect was not caused simply by brain damage or other obvious artifacts. Damage at the tip of the cannula was little and extended less than 1 mm from the tip. We determined that in this region of the MRF and in the area of hippocampal perfusion, only 1 to 2 percent (by weight) of wet brain tissue was soluble protein (*7*). Consequently, at least 100 to 200 mg of wet tissue (a 4- to 5-mm cube), much more than the amount of tissue damaged by the cannula, would be required to obtain 2.0 mg of soluble protein.

If the presence of protein in the perfusate was due to cellular damage, the concentration would be highest immediately after the cannula was inserted. The results do not support this interpretation; the protein content in several experiments was lowest immediately after insertion (Fig. 1). Further, when a cannula was removed and reinserted at a low or high point of the cycle, the concentration of the next sample reflected a continuation of the cyclic trend; reinsertion did not result in higher concentration in the next sample. Thus, these results suggest that the amounts of protein in the perfusates

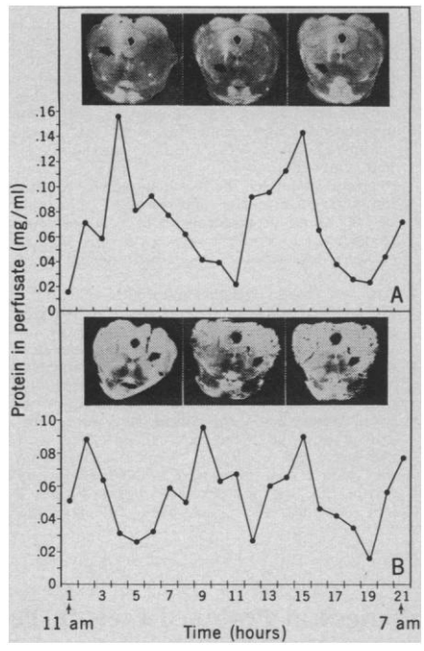

Fig. 1. Time-dependent changes in the protein concentration of perfusates collected by means of a push-pull cannula positioned in the medial reticular formation. Each graph gives data from one cat; the inset above shows the position of the cannula in sequential 50-μm sections. The size of the lesion closely corresponds to the actual diameter of the cannula. At the end of the cannula tip, little tissue damage is apparent.

were not related to tissue damage, but seemed to reflect time-dependent cyclic variations. However, we cannot exclude the possibility of some minor contribution from tissue damage or related causes.

In several experiments we investigated the characteristics of the proteins collected in MRF perfusates. As assessed by polyacrylamide gel electrophoresis (*8*), the perfusates contained several proteins, some of which were different from those in the total soluble protein fraction in samples of tissue from the brain region (Fig. 2). These

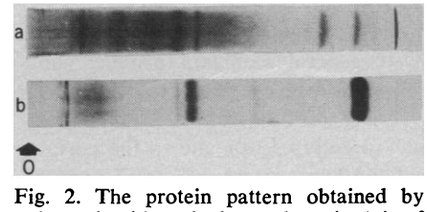

Fig. 2. The protein pattern obtained by polyacrylamide gel electrophoresis (a) of soluble protein extracted from samples of medial reticular formation and (b) of protein in perfusates from this brain area; *O*, origin.

findings provided further evidence that brain damage alone could not account for the protein content of the perfusates since cellular damage would be expected to release all soluble protein.

Ultrafiltration analyses (*9*) indicated that approximately 80 percent of the proteins were of molecular weight greater than 10^4 and at least 60 percent were greater than 5×10^4. The presence of protein was verified by incubating the perfusates in proteolytic enzymes (Bromalein, 50 μg/ml) at 37°C for 24 hours, which produced components of low molecular weight. Little or no degradation occurred when the perfusates were incubated (37°C for 24 hours) without proteolytic enzymes, which indicated that substances causing degradation were absent from the perfusate.

To examine the possibility that serum proteins were in the perfusates, we analyzed the perfusates by immunoelectrophoresis (*10*). In some samples there was no evidence of serum proteins, while in others a few at negligible concentrations were noted. Thus, the proteins in the perfusates appeared to be a distinctive class from the region of the MRF sampled.

These results indicate that proteins can be obtained from the extracellular space of the brain by means of a push-pull cannula. The protein concentration rises and falls over a 24-hour period. The fact that the protein concentrations are high, that they are not influenced by cannula insertion, and that only certain proteins are obtained suggests that the proteins are not released from cells simply because of tissue damage but, rather, reflect cyclic variations in extracellular proteins.

We investigated the possibility that the variations in protein concentration in the perfusates represented some active physiological process. In two cats, protein levels decreased rapidly after administration of a lethal dose of pentobarbital sodium. Our findings strongly suggest that the variations in protein levels are associated with changes in some physiological state. In the cat the periods of cyclic changes in protein levels in the perfusates are similar to those of the sleep-waking cycle. In view of other evidence suggesting a relationship between sleep states and protein (*11*), we compared the protein concentration of perfusates from waking periods with those obtained during rapid eye movement (REM) sleep

(*12*). In 10 of 12 paired MRF samples collected, the concentration of protein during REM sleep was significantly higher than that during awake periods (paired t-test = 3.94, $P < .001$). Furthermore, the increases of hippocampal proteins were always associated with increases in REM sleep. These results are consistent with the possibility that neuronal activity in the MRF and hippocampus during REM sleep is associated with high concentrations of extracellular proteins. These cyclic changes may simply reflect an increase in cell firing, which generally increases in REM sleep (*13*), or may indicate a special function of the proteins related to awake and sleep states.

An essential question concerns the source of the perfusate proteins. These proteins might be released from synaptic endings in a manner similar to that for dopamine-β-hydroxylase (*5*) or other products of the exocytosis process. Polypeptide modulators or neurotransmitters may be present. Alternatively, the proteins may be general secretory products of neurons or glial cells. In view of the amount of protein obtained, highly active synthetic processes seem implicated. These proteins probably arise from a variety of sources. The significant point, however, is that the protein concentrations vary in relation to REM state. Our studies provide further evidence that the push-pull cannula technique is well suited for the examination of the brain's extracellular environment. This procedure should further our understanding of the neurochemical basis of behavioral states.

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9 October 1974

Meaning in Visual Search

Abstract. *Viewers briefly glimpsed pictures presented in a sequence at rates up to eight per second. They recognized a target picture as accurately and almost as rapidly when they knew only its meaning given by a name (for example, a boat) as when they had seen the picture itself in advance.*

When we look around, our glance shifts rapidly from point to point. The average fixation lasts a mere ⅓ second. Moreover, when the observer is moving through an unfamiliar environment, each such glance may contain something new. We know that when novel scenes (pictures) are viewed at that rate, half of them do not even look familiar a few minutes later (*1*). Each glance is thus too brief to assure memory for what is seen. What other function (*2*) might such brief fixations serve? When the viewer can anticipate either what is important to see or what he is likely to see, a brief glimpse may be sufficient to confirm or refute that expectation.

Two questions were addressed in the study reported here. (i) Can an observer detect an expected scene even when it is presented so briefly that it would not otherwise be remembered? (ii) If so, what sort of advance information about a scene is required for the observer to spot it? If a viewer knew exactly what the target of his search looked like, he might select it by making a direct visual match. However, if he had only general information about the target (its meaning), each potential target would have to be recognized and categorized before selection. Such a search might be expected to proceed more slowly and less accurately than one based on visual appearance per se (*3*). In the study reported here observers were able to pick out an anticipated scene from a set of others presented at rates even higher than those of normal eye refixations, rates at which memory for unanticipated scenes is very poor. Even more surprisingly, foreknowledge of meaning in the form of a general name permitted as accurate and almost as rapid selection as foreknowledge of exact appearance. These results suggest that we can scan our environment in brief glimpses, looking not only for particular visual patterns, but for their meanings.

A succession of rapid glances around the environment was simulated by presenting observers with a sequence of photographs of various scenes and objects (*1*). One practice and eight test sequences of 16 color pictures were shown on an L-W cine projector to two groups of 24 college students. The observer was instructed to look for a particular picture; if he saw it, he responded by pressing a lever that stopped the projector. In one group, the observer was shown the target picture before viewing each sequence. In the other group, the observer was only given a name for the picture he was to look for (for example, a boat, two men drinking beer, a child and butterfly). The names were brief descriptions of the main objects or events in the scene; colors and shapes were never specified directly. In all other respects, the procedure was identical for both groups.

Each observer viewed the practice