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"NEUROPHYSIOLOGICAL ESTIMATES OF HUMAN PERFORMANCE CAPABILITIES IN AEROSPACE SYSTEMS"

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W. R. Adey, M.D.
Professor of Anatomy and Physiology

Space Biology Laboratory
Brain Research Institute
University of California, Los Angeles

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13. ABSTRACT

This report is a multiphase research effort which involves a number of very distinct and separate projects, some of which are listed below.

1. Neurophysiological Correlates of Tasks at the Limit of Operator Capability
2. Totally Implanted Telemetry Devices; Underwater Telemetry
3. Telephone Telemetry
4. Theory and Methods of Fuzzy Clustering Techniques
5. Investigations of CNS and Endocrine System in the Chimpanzee
6. Patterns of Diurnal Rhythms in Urinary Metabolites and
7. Electrolytes in Men with Spinal Cord Injury
8. Fluorescence Spectroscopy and Biological Labelling
9. Effects of Modulated VHF Field on Brain and Behavior
10. Computer Studies of EEG in Normals and Children with Reading Difficulties

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PROGRESS REPORT

JAN BERKHOUT

1. Neurophysiological Correlates of Tasks at the Limits of Operator Capability: In the past year we have recorded 4 channels of EEG, 2 of EOG and one of EKG data from 48 law enforcement officers driving an instrumented patrol car on a closed course at very high speeds. This data is presently being analyzed to determine what physiological patterns precede appropriate vs. inappropriate vehicle control maneuvers, and whether such patterns can be used to predict a subject's propensity to incur accidents in law enforcement service.

   We have prepared an inventory of all taped EEG data in our laboratory which was recorded during perceptual or psychomotor performance tasks. This mass of material will be segregated according to performance levels simultaneously achieved, and submitted to a discriminant analysis procedure (DISCAN) designed to identify any neuroelectric patterns consistently accompanying good or poor performance. Since the pool of data to be used exists in a wide array of formats, a considerable programming effort is being made to increase the flexibility and generalizing power of the DISCAN procedure. Eventually it will be feasible to distinguish patterns occurring across a variety of electrode montages and spectral parameters. We have plans to incorporate those patterns discovered to be associated with good performance into feedback-mediated enhancement-of-activity studies leading to individual volitional control of optimally alert, performance oriented brain states.

2. EEG Studies of Human Subjects in a High +Gz Gravitational Field: We have completed a series of recordings at the SAM centrifuge at Brooks AFB, San Antonio, in which 14 subjects were exposed to a balanced program of $+4\frac{1}{2}$ and +6 Gz centrifuge runs. Preliminary analysis of this data suggests that some changes in the concurrent EEG can be discerned during the actual runs, despite the high intensity of tifacts, but that such changes do not indicate any persistent pathological activity as a result of +Gz exposure and do not persist into post-exposure epochs. Further studies on EEG accompanying rapid deceleration (-Gx) are presently under design in collaboration with the Naval Air Development Center, Crew Systems Branch, Warminster, Pennsylvania.

J. R. ZWEIZIG

Totally Implanted Telemetry Devices:
Life tests have been made on implantable micropower telemeters over a period of thirty days of continuous operation. Field intensity, carrier frequency and deviation sensitivity were recorded at hourly intervals during the weekday hours of the test period. Battery voltage varied monotonically from 1.562 to 1.532 over the period of observation. The silver oxide cells are rated at sixty milliampere-hours, weigh on the average 1.2 grams and have been utilized in totally implanted telemetry

because of their stability and high power to volume and weight ratio.

The use of a small diameter loop antenna in connection with a high frequency ferrite core has proven useful in gaining maximum power transfer to the receiving antenna. The high Q of the resonant circuit is maintained by the ferrite core even when the appliance is surrounded by body fluid and tissue.

High Impedance Headstage Amplifier:
A differential Microelectrode preamplifier was constructed for use in recording unit spikes in the respiratory center of the cat. Leakage currents of less than 10^{-11} amperes were obtained with a frequency response from DC to 60 K Hz. Input resistance was in excess of 10^{11} ohms and noise with high frequency response limited to 6 K Hz was less than 25 uv peak to peak. Excellent spike data has been recorded using etched tungsten wire electrodes. The headstage operates with a gain of 10 and has a supply current requirement of less than 200 microamperes.

EEG Electrode Harness:
Field recording of EEG under conditions involving stress continues to be of major interest to the laboratory. Subject safety as well as convenience in the experimental situation require an easily appliable set of sensors. In addition the subject should not be hampered in his movements by connection leads to the recording apparatus. IRIG subcarriers utilizing frequency multiplexing afford a convenient and accurate method of telemetering the data. One recent application of the method is in connection with stressful driving in motor vehicles.

Underwater Telemetry from Free-Swimming Divers:
Recently EEG data from a diver fifty feet below the surface of the ocean has been recorded at a range of approximately 30 ft from the receiving antenna. Transmission through the sea water channel is by means of a new technique known as return current density communication. As is well known, high frequency radio signals are attenuated rapidly in sea water. For this reason transmission from free swimming divers has been lacking due to the limitations of conventional telemetry equipment. High frequencies are also attenuated using return current density, however IRIG channels 1 through 8 appear suitable for use. Attenuation of the carrier current density due to skin effect increases with distance but allows a reasonable working range for the diver at these relatively low frequencies. Extension to frequencies higher than 3 K Hz appears doubtful at present based on phase shift measurements in a sea water channel. The information bandwidth for these channels is adequate for EEG, EOG, and a restricted frequency range EKG.

Paper in Preparation:

with W. R. Adey, J. Hanley, P. Hahn and A. Pilmanus
"EEG Monitoring of Free-Swimming Diver at a Working Depth of Fifty Feet"

Papers Published:
Dymond, A.M., J. R. Zweizig, P. H. Crandall, J. Hanley,

"Clinical Application of an EEG Radio-Telemetry System"
Proc. of the Eighth Annual Rocky Mountain Bioengineering Symposium
May 3-5, 1971, Colorado State University, Fort Collins, Colorado

J. Hanley, W.R. Adey, J.R. Zweizig and R.T. Kado
"EEG Electrode-Amplifier Harness"
Electroenceph. clin. Neurophysiol., 1971, 30: 147-150.

JOHN HANLEY

1. Telephone Telemetry:
In addition to the use of telephone telemetry described in the previous report, we are now carrying out epilepsy screening and EEG survey for other abnormalities in a medically indigent Mexican American community in East Los Angeles. This community consists of a barrio of 350,000 people in which the incidence of epilepsy is about 5 times that of an equivalent middle-class community in another area of Los Angeles. The so-called Chicano group has a culturally determined fear of hospitals and do not go to the available (though already overburdened) County Hospital. In cooperation with the County facility, we are transmitting EEGs from a small neurological clinic established in the barrio to USC-County Hospital and to UCLA. Evaluation of the signal is immediate, and interpretation telephoned directly back to the neurologist either through the same system or over another telephone. Early studies reveal a much higher incidence of abnormalities than in the general population.

Within the UCLA hospital, we have extended monitoring capabilities to the anesthesiologist during open-chest cases. Patients EEGs are continuously monitored during the surgery, following a base-line recording. Telemetry allows collection of data on a non-interference basis and the surgeon is not hampered. A number of studies are resulting from these recordings, including neurophysiological correlates of particular anesthetics during extracorporeal circulation. Collaboration with the Pharmacology department is also permitting simultaneous acquisition of blood gases.

2. Eye Movement Studies on Post-Opthalmological Surgical Patients (with P.M. Hahn):
It is common practice to patch the eyes of patients following eye surgery with the object of resting the eye. In an attempt to see if this actually is achieved, we have been telemetering the electro-oculogram (EOG) from these patients under conditions of both eyes patched, one eye patched, and neither eye patched. Telemetry is ideal for this experiment: The transmitter takes up little space and the signal is sent to a small receiver in the patients room. We are also experimenting with recording the signal on inexpensive cassettes for later playback. Early results indicate that with only one eye patched, eye movements actually increase. To achieve rest, both or neither seem more effective.

3. EEG Studies of Cannabis Sativa Inhalation in the Human (with P.M. Hahn):
We are continuing our earlier studies of cannabis sativa in man. Both hard wire and telemetry recordings are taken before and after use, at

present in two laboratory settings. Thus far, we have 1 hr recordings post-drug use but plan for up to 4 hrs of follow up. These studies will be contrasted with the use of the drug in more usual environments. The EEG is stored on magnetic tape for computer analysis. Our earlier results indicated dramatic discrimination between pre and post use of drug.

4. Computer Studies of EEG in Normals and Children with Reading Difficulties (Dyslexia) (with B. Sklar):
We have attempted to differentiate between dyslexic children, defined here as reading difficulties in children not due to mental retardation, and age-matched controls who either do not have such problems or who easily surmount them. Though the time domain recordings of these groups do not appear different, discriminant analysis programs of their spectral transforms yield consistent differences between dyslexic and normal children in the rest state, during mental arithmetic tasks, and during reading both word lists and text. 25 children have been studied so far with better than 85% categorization. The discrimination programs have been utilized on a large computer initially, and then programs designed for smaller computers, with significant cost savings and time economies with essentially as high a success in discrimination. These studies may aid in a different approach to the teaching of reading in these children, as well as in the design of a special purpose device as a diagnostic aid for the pediatric Neurolcgist. Other learning difficulties encountered in many situations may also yield correlates of such difficulties and provide valuable screening techniques.

Meetings Attended:

Nov. 1970	Annual Conference on Engineering in Medicine and Biology. (Program Chairman elect.) Washington, D.C.
Jan. 1971	4th Winter Conference on Brain Research. Snowmass-in-Aspen, Colorado. (Panelist)
Sept. 1971	2nd Annual Symposium on Non-linear Estimation Theory. San Diego, California. (Panelist) (IEEE and AFOSR supported meeting.)
Sept. 1971	American EEG Society Annual Meeting. Bloomington, Minnesota. (Invited Workshop participant.)
Nov. 1971	24th Annual Conference on Engineering in Medicine and Biology. Las Vegas, Nevada. (Program Chairman)

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1) Hanley, J., Adey, W.R., Zweizig, J.R., and Kado, R.T., "An EEG electrode-amplifier harness", EEG clin. Neurophysiol., 30: 147-150, 1971.

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3) Sklar, P., and Hanley, J., "A multi-fontal alphabet for dyslexic children", J. Learning Disabilities (In press).

4) Nirenberg, L.M., Hanley, J., and Stear, E.B., "EEG motor signal tracking with an adaptively designed phase-locked loop", IEEE Trans. on Bio-Med. Engnr., BME-18(6), Nov. 1971.

5) Hanley, J., Rickles, W.R., Crandall, P.H., Walter, R.D., Lessman, R., Walter, D.O., and Zweizig, J.R., "Automatic recognition of EEG correlates of behavior in a chronic schizophrenic patient", Amer. J. Psychiat.

6) Rossi, G.F., and Hanley, J., "Changes in responsiveness to visual stimuli of cortical and thalamic 'non-specific' systems during wakefulness and sleep in man", in: Psicofysiologia del Sonno e del Sogno, Vita and Pensiero (eds.), Milano, 32-39, 1970.

7) Hanley, J., Rickles, W.R., Crandall, P.H., and Walter, R.D., "Spectral characteristics accompanying deep spiking in a patient with chronic schizophrenia", EEG Clin. Neurophysiol., 28: 90, 1970.

ENRIQUE H. RUSPINI

Research:
Work has continued steadily on the theory and methods of fuzzy clustering. New applications to pattern recognition were developed and studied. Applications also continued on the study of EEG using fuzzy clustering techniques. These studies are centered around sleep staging and discrimination of behavioral states. The possibility of applying fuzzy clustering techniques for the study of decision-making processes is presently being investigated.

Studies have continued on techniques for system identification and modeling for biological systems. Special attention has been given to problems and in the renewal theory study of nerve nets. Techniques developed for the solution of these problems have proven to be useful in other areas of science like radiative transfer and hydrology. Potential applications of some of the methods developed include the study of the problem of elastic behavior of the skull (head injury) and determination of generations of EEG using multiple surface measurements.

Research is also proceeding in the determination of optimal strategies for the computer augmentation of bio-feedback responses.

Publications:

(Submitted, published or in press April 1970 - October 1971)

1) Kalaba, R. and Ruspini, E.H., Invariant Imbedding and Radiative Transfer in a Homogeneous Cylindrical Medium, J. Quant. Spect. Rad. Transfer, 11, 1063-1074, 1971.

2) Ruspini, E.H., Numerical methods for fuzzy Clustering, Information Sciences, 2: 319-350, 1970.

3) Larsen, L.E., Ruspini, E.H., McNew, J.J., Walter, D.O., and Adey, W.R., A test of sleep staging systems in the unrestrained chimpanzee, in submission.

4) Larsen, L.E., Ruspini, E.H., McNew, J.J., Walter, D.O., and Adey, W.R., Classification and discrimination of the EEG during sleep, article in book "Automation of clinical EEG," edited by P. Kellaway and Peterson, in press.

5) Kalaba, R. and Ruspini, E.H., Radiative transfer in a homogeneous cylindrical atmosphere with a large radius, in submission.

6) Kalaba, R. and Ruspini, E.H., Computation of the source function for cylindrical atmospheres of small radius, in submission.

7) Buell, J., Kagiwada, H., Kalaba, R., Ruspini, E.H. and Zagustin, E., Exact solution of a system of dual integral equations. University of Southern California, Department of Electrical Engineering Technical Report No. 70-49, 1970; Int. J. Engnr. Sci., in press.

8) Ruspini, E.H., Invariant imbedding and elliptic equations. (Abstract) Proceedings of Fourth Hawaiian International Conference on System Science (invited paper), Honolulu, Hawaii, 1971.

9) Buell, J., Kalaba, R., Ruspini, E., Yakush, A., A program for identification of linear systems, Computer Programs in Biomedicine, in press.

10) Ruspini, E.H., New experimental results in fuzzy clustering, in submission.

11) Ruspini, E.H., Applications of fuzzy clustering to pattern recognition, in submission.

12) Kalaba, R., and Ruspini, E. "Invariant imbedding and a matrix integral equation of neuronal networks," Mathematical Biosciences, in press.

13) Larsen, L.E., Ruspini, E.H., McNew, J.J., Walter, D.O., and Adey, W.R., Classification and discrimination of the EEG during sleep, article in the book "Automation of clinical EEG" edited by P. Kellaway and Peterson, in press.

14) Ruspini, E.H., Optimization in Sample Description: Data Reduction and Pattern Recognition using Fuzzy Clustering. (Invited paper) presented at the Pattern Recognition Workshop of the Joint National Conference on Major Systems, Anaheim, California, October 1971. Abstract to be published in Systems, Man and Cybernetics.

15) Ruspini, E.H., New Cluster Analysis Techniques: Fuzzy and Probabilistic

Clustering and some of their Applications, Fifth Hawaii International Conference on System Science (Subconference in computers in biomedicine), Honolulu, Hawaii, January 1972. Proceedings.

16) Ruspini, E.H., and Distefano, J.N., On a class identification problems in Hydrology, Fifth International Conference on System Science, Honolulu, Hawaii, 1972. Proceedings.

Meetings Attended:

Fourth Hawaii International Conference on System Science, Honolulu, Hawaii, January 1971. (Invited paper presented)

Gordon Research Conference on Theoretical Biology and Biomathematics, Andover, New Hampshire, June 1971.

Pattern Recognition Workshop of the Joint National Conference on Major Systems, Anaheim, California, October 1971. (Invited paper presented)

Fifth Asilomar Conference on Circuits and Systems, Monterey, California, November 1971. (Session Chairman and Organizer)

IRENE SABBOT

1. Investigation of the CNS and Endocrine System in the Chimpanzee:
During the past year our efforts have been directed toward completion of the majority of the projects involving the chimpanzee. The main reason for this was the transfer of Dr. J. McNew from UCLA to the University of Tennessee at Chattanooga, due to decreases in funding for this project. Thus, this portion of the report shall also incorporate the work performed by Dr. McNew.

Effect of 2-day food deprivation stress on 24-hour urine excretion values:
The study was designed to determine 24-hour urinary excretion values and note the changes in urinary values induced by the stress of 2-day food deprivation. A manuscript was submitted to the American Journal of Physiology and should be in press. Paper (1) of I. Sabbot publications list.

The sleep-cycle and subcortical relations in the unrestrained chimpanzee:
Study of nocturnal sleep in the young unrestrained chimpanzee in his cage environment using a 4 channel biotelemetry system. The sleep cycle was described by use of computer analysis techniques and visual inspection. The data acquired has contributed to the preparation of several manuscripts (Freemon, McNew and Adey, 1969-1970; Larsen et al, 1970 and in press; McNew, Howe and Adey, in press).

Biorhythms in urinary excretion of hormones, metabolites and electrolytes and the sleep-wake cycle in the unrestrained chimpanzee:
The study was designed to determine the relationship between the sleep-wake cycle and the rhythmicity in urinary excretion patterns in chimpanzees

under home cage environmental conditions. Analysis of the CNS data of the sleep-wake cycle was completed and the results incorporated into three manuscripts (see McNew publications; Freemon, McNew and Adey, 1969, 1970, and in press). Urinary analysis on the four experimental "runs" are completed. A manuscript has not as yet been prepared.

30-day isolation stress and its effects on CNS and metabolic values and biorhythms in the unrestrained chimpanzee:
Central nervous and metabolic values and biorhythms were studied in an unrestrained chimpanzee subjected to 30 days of isolation. The animal received 10 days of 12 hours of light and 12 hours of dark, then 10 days of continuous light, followed by 10 more days of 12 hours of light and 12 hours of dark.
Sleep-Wake Cycle. The circadian sleep-wake rhythm and the Wake and Sleep phases of this rhythm during entrained and free running conditions were analyzed in terms of duration. The awake and nonREM sleep and REM sleep stages were also analyzed. In addition, the mean duration of the sleep cycle of the sleep phase was computed. Under the entraining conditions of the first and last ten days, the chimpanzee's sleep-wake cycle was 24 hours. During the continuous light period of isolation, the mean duration of the circadian sleep-wake rhythm was 24.8 hours. The significantly longer free running sleep-wake rhythm may be attributed to the animal spending more time in the awake and REM sleep stages. The animal's sleep cycle during the sleep phase of the sleep-wake rhythm averaged about 100 minutes during both the entrained and free running conditions. (McNew et al, Aerospace Medicine, in press).
Excretion parameters and their rhythms. The study consisted of 49 urine collection days (14 days pre-, 5 days-post, and 30 days of isolation), and then of 10 days in the home cage. Dietary intake, urine and fecal data were obtained. The effect of isolation on various excretory parameters was studied.

Urine samples were analyzed for volume, osmolarity, creatinine, creatine, urea-N, 16-hydroxy corticosteroids, VMA, calcium and inorganic phosphorus. One way analysis of variance performed on the urinary excretion parameters showed all except creatinine excretion to vary significantly during various periods of the study. The changes observed in calcium and phosphorus were highly significant.

For the 30 days of isolation, the calcium balance remained positive while the phosphorus balance became negative after the first 10 days of isolation and progressively more so as the isolation continued. During isolation, the calcium to phosphorus intake ration (Ca/P) did not vary, while the Ca/P excretion ratios (urine, fecal, urine + fecal) progressively decreased. The data suggests that the calcium to phosphorus excretion ratio might serve as a physiological stress indicator of Selye's adaptation syndrome (period of resistance). (Sabbot et al; Aerospace Medicine, in press).
Urine volumes and voiding times were recorded every hour beginning 14 days prior to isolation, ending 6 days after isolation, and approximately 2 months later for 10 days as a control. Observed during most periods of the experiment were 1) clear circadian micturition rhythms with the voiding

peak occurring immediately after the subject awoke and 2) urine flow rhythms with the maximum volume voided in the morning hours. A 24-hour rhythm was seen when the subject was entrained to 12L:12D treatments and a 24.8-hour rhythm when he was exposed to continuous light. A possible underlying 24-hour micturition rhythm was also seen during the continuous light period.

Distorted rhythm curves indicating possible stress were obtained for the pre-isolation adaptation period and the initial period of isolation. As time passed, the curves were more like the controls, perhaps indicating a decrease in stress.

The urine volume data also indicated stress during the pre-isolation period and decreasingly so in the succeeding periods of isolation. During the 6 days after isolation was broken, an increase in stress was also indicated. (Hoshizaki et al, Aerospace Medicine, in press).

The data obtained from the urine analysis has been punched on IBM cards and subjected to a newly written computer analysis technique of Mr. E. Ruspini's (HUG of Hypothetical Urine Flow). Mr. Ruspini is also in the process of writing up another program which is going to search for periodicities in our data blocks and should be of great value in our biorhythm studies.

The sleep-wake cycle and the biochemical investigations of urinary excretion patterns in the couch-restrained, catheterized chimpanzee: Couch restrained, catheterized chimpanzees were studied for 10 successive days. Electrophysiological measures of the sleep-wake cycle were recorded and urine fractions were collected every 12 minutes for the duration of the experiment. Three animals were used in the study which was successful for the 10-day duration in 2 animals.

The preliminary results of some of the analysis have been reported earlier. To date we have completed the analysis of volume, pH and osmolarity on all 12 minute samples. This data, along with other results shall be placed on IBM cards and subjected to the periodicities program mentioned above. The EEG records were taken by Dr. McNew, who hopes to analyze at least one 10-day study into sleep-wake stages, so that a comparison between the sleep-wake and excretory patterns can be made.

2. Patterns of diurnal rhythms in urinary metabolites and electrolytes in men with spinal cord injury (high cervical and thoracic). Study with Dr. Adey.
An alteration in sleep patterns was found by Dr. Adey et al, in patients with high cervical lesions the most marked occurring with a long time duration since the onset of lesion. Other investigators have reported losses of electrolyte rhythms (Na and K excretion) in the "acute" phase since injury (i.e. up to 8 months) vs. "chronic" (i.e. over 8 months since lesion). This study was undertaken to investigate excretory rhythms in both acute and chronic quadriplegics (6 subjects) as well as paraplegics (5 subjects). Urine specimens were collected by means of a

fraction collector and pooled to obtain hourly values. The urine analysis included: volume, osmolarity, creatinine, 17-hydroxysteroids, VMA, calcium, phosphorus, sodium and potassium. A difference between the acute and chronic patterns was observed, especially in the corticosteroids, also in the amplitude of the response. Preliminary results indicate changes in electrolyte excretion. The data is currently being put on IBM cards in order to be analyzed for rhythmic patterns by the periodicities computer program mentioned above. A manuscript shall then be prepared.

3. Patterns of urinary metabolites in human circadian displacements: Dr. Berkhout's study of Desynchronosis. Results of urine analysis were reported previously by Dr. Berkhout. Biorhythms in sodium and potassium excretion in humans under simulated time-zone shifts of short duration remain to be examined when time permits.

4. Investigation of distribution of cations into brain area: Study involving radioactive tracers investigating the uptake and distribution of cations (^{45}Ca, ^{47}Ca, ^{24}Na, and ^{42}K) into brain areas of various species and the influence of amino acids and certain drugs on same. The work will eventually include autoradiographic demonstration techniques for the cations, calcium in particular. Preliminary experiments shall begin hopefully within the next two months (as soon as the radioisotope permit is obtained).

Publications:

Publications which have resulted from this work:

a) I. Sabbot and the Endocrine System

1) McNew, J.J., Sabbot, I.M., Mandell, A.J., Spooner, C.E., Marcus, I., and Adey, W.R. Urinary excretion values in two-day food deprived, unrestrained chimpanzee, Am J. Physiol., 1971, In press.

2) Sabbot, I.M., McNew, J.J., Hoshizaki, T., and Adey, W.R. Calcium and phosphorus excretion during short term stress and prolonged stresses in the unrestrained chimpanzee. Aerospace Med. Assoc., Proceedings of Ann. Sci. Meeting, 17-18, 1971.

3) Hoshizaki, T., MdNew, J.J., Sabbot, I., and Adey, W.R. Effects of 30 days of isolation on the periodic micturition patterns of an unrestrained chimpanzee, Aerospace Med. Assoc., Proceedings of Ann. Sci. Meeting, 244-245, 1971.

4) Hoshizaki, T., McNew, J.J., Sabbot, I., and Adey, W.R. Micturition patterns of an unrestrained chimpanzee under entrained and free running conditions, Aerospace Med., 1971, In press.

5) Sabbot, I., McNew, J.J., Hoshizaki, T., Sedgwick, C., and Adey, W.R. Effect of a 30-day isolation stress on calcium, phosphorus and other excretory products in an unrestrained chimpanzee, Aerospace Med., 1971, In press.

b) J.J. McNew et al:

1) McNew, J.J., Kado, R.T., Howe, R.C., Zweizig, J.R., Adey, W.R. Telemetry studies of sleep in the unrestrained chimpanzee, IEEE Cat. No. 68-C-8-NTC, 374-380, 1968.

2) Freemon, F.R., McNew, J.J., Adey, W.R. Sleep of unrestrained chimpanzee: Cortical and Subcortical recordings, Exp. Neurol. 25: 129-137, 1969.

3) Freemon, F.R., McNew, J.J., Adey, W.R. Sleep of Unrestrained chimpanzee: Differences between first and last REM periods, Folia Primatologia, In press.

4) Larsen, L.E., Walter, D.O., McNew, J.J., Adey, W.R. On the problem of bias in error rate estimation for discriminant analysis, Pattern Recognition, In press.

5) Stoller, D.F., Kinney, J.P., Burson, R.C., McNew, J.J. Apparatus for collection of individual micturition samples from unrestrained primates, Physiol. Behav. 6: 91-93, 1971.

6) McNew, J.J., Howe, R.C., Adey, W.R. Sleep cycle and subcortical-cortical EEG relations in the unrestrained chimpanzee, Electroenceph. Clin. Neurophysiol., 1971, In press.

7) Larsen, L.E., Ruspini, E.H., McNew, J.J., Walter, D.O., and Adey, W.R. Test for sleep staging systems in the unrestrained chimpanzee. Submitted for publication.

8) Freemon, F.R., McNew, J.J., Adey, W.R. The sleep cycle of the chimpanzee. Submitted for publication.

9) McNew, J.J., Burson, R.C., Hoshizaki, T., and Adey, W.R. The sleep-wake cycle of an unrestrained isolated chimpanzee under entrained and free running conditions, Aerospace Med., 1971, In press.

Meetings Attended by I. Sabbot, J.J., McNew and T. Hoshizaki

Aerospace Medical Association, 1971 Annual Scientific Meeting
Houston, Texas, April 26-29, 1971.

Papers Read:

"Calcium and Phosphorus Excretion During Short Term and Prolonged Stresses in the Unrestrained Chimpanzee" by I. Sabbot.

Other Papers Presented at Same Meeting:

"Effect of Continuous Light on the Sleep-Wake Cycle of an Unrestrained Isolated Chimpanzee" by J.J. McNew.

"Effects of 30 Days of Isolation on the Periodic Micturition Patterns of an Unrestrained Chimpanzee" by T. Hoshizaki.

HAROLD LYONS

Fluorescence Spectroscopy and Biological Labelling:
The principal effort of the past year has been devoted to chemical synthesis, analysis and optimization of rare earth chelate - protein conjugates for fluorescence labelling. By reference to earlier reports, it will be recalled that such fluorochromes provide sharp line emission, and that interfering tissue fluorescence and scattering can be eliminated by filters or by the frequency-phase-resolved technique previously developed. Emphasis has been placed on labelling antibodies, so that specific sites, such as neuronal membranes, could be selectively labelled by antibody-antigen complexing. Such a fluorochrome marker would then be used as a probe of the environment around the marker, within a few angstroms, by studying environmentally-induced changes in the spectrum and decay time of the markers.

A successful chelate for labelling antibodies was developed using 4, 7-diphenyl-1, 10-phenanthroline-X, X-disulfonic acid, disodium salt, converted to the sulfonyl chloride to conjugate with protein. As already reported, this chelate using europium works well in organic solvents, but is quenched in aqueous solution, unless precipitated. An enormous number of experiments have been carried out to make a mixed chelate conjugate, which would fluoresce brightly in aqueous solution. A sampling from this work will be reported here.

The best second ligand for fluorescence of the mixed chelate in water, was found to be TTA, tris (4, 4, 4-trifluoro -1-(2-thienyl)-1, 3, butanediono) europium. With this mixed chelate, a sharp principal emission line at room temperature, with a line width of about 35 angstroms was obtained. An environmental shift of this line of a few angstroms could be measured.

The fluorescence of the mixed chelate was found to fade. Various lines of evidence were adduced, leading to the conclusion, that the TTA displaced the phenanthroline ligands, since their equilibrium constants were greater. In a continuing program, research is being carried out, by immunoelectrophoresis techniques, both to show that the labelled antibodies have not been denatured and that the fluorescence fading can be prevented by fixing the antibody-antigen complex in place, so that collisions between TTA-carrying proteins is eliminated.

Another difficulty investigated has been the development of lattices by the labelled antibodies causing large aggregates, which precipitate out of solution. This is caused by two effects; first, there are two sulfonyl chloride groups per ligand and second, chelating with europium binds three or four proteins together, depending on whether tris or tetrakis chelates are made. The molecules thus "join hands" to make lattices. The first difficulty can be cured by using a ligand with only one

protein-conjugating group. So far it has not been possible to obtain such a ligand commercially. Synthesis of such a compound may be necessary. The second difficulty can be largely cured by using a mixed chelate, in which one ligand only carries the protein-conjugating functional group, and the others are exactly the same except for the lack of this group. The extra conjugating is thus eliminated, while the equilibrium constants of all the ligands are essentially the same, so a stable configuration results. It was found that the original sulfonic acid ligand could not be used as a "neutral" ligand, being incompatible with sulfonyl chloride. However, other phenanthroline ligands were obtained without the sulfonic acid groups.

Another partial difficulty was discovered, which might also be applied in a useful way in some cases. It was discovered that antibodies pick up europium ions directly, without the use of phenanthrolines, and these are, of course, water soluble and fluoresce well in aqueous environment. The nature of the finding is not fully understood, but is strong enough to cause difficulties in separating labelled antibodies from europium by chromatography. The binding seems, however, to be too weak to provide reliable and permanent fluorchrome labelling under all conditions, including such cases as continuous washing. The covalent binding using the chelate is much stronger. This direct ion labelling makes the interpretation of some techniques difficult, and has resulted in much effort being expended on making a chelate first and conjugating it second, to avoid exposure of the protein to free europium. So far it has not been possible to use this method, either by making a chelate and then converting to sulfonyl chloride or by chelating the sulfonyl chloride ligands and then attempting conjugation. The pH, solvent and other conditions needed for chelating first, apparently destroy the sulfonyl chloride group. These difficulties also pose problems in accurate stoichiometric preparation of the antibody labels, which would have been avoided if chelation could be performed first. Nevertheless, studies are being carried out to see if this simple method of labelling antibodies with europium chelate is applicable to staining slides of pathogens, tissue, etc. for study by means of the fluorescence microscope, where fixing could be employed to prevent difficulties from weak binding. Similarly, the use of phenanthroline alone, rather than in a mixed chelate with TTA, is being investigated for application to the fluorescent antibody technique; thus, tissue, bacteria, etc., could be dehydrated first and then a non-quenching solvent used to give bright fluorescence. Alternatively, the preparation could be developed with TTA even in aqueous solution, as long as excess TTA is washed away, and the substrate is prevented from exchanging TTA ligands with phenanthroline.

2. Continuing Program:

The applications and difficulties mentioned above will be further studied and refined. Emphasis will be given to further synthesis and analysis studies, application to staining pathogens and bacteria, and if results warrant, to the fluorescent staining of clones of neurons to study neuronal membranes. Development of better chromatographic and other needed instrumentation will be carried out if funds allow.

Publications:
It is planned to finish preparation for publication of several aspects of the above work, when present studies relative to denaturation and the fixing of substrates is completed.

BARBARA BYSTROM

1. Correlated Electron Microscopic and Electrophysiological Studies on Alterations Frought About by Injections of Enzymes and Antibodies into Lateral Geniculate Bodies of Cats:
Cortical controls and samples from the left and right lateral geniculate bodies of four cats and left and right hippocampal regions of one cat used in electrophysiological studies (Dr. A. Costin) have been embedded for electron microscopy. Sections are now being processed from triplicate blocks of each tissue for neurocytological comparison.

2. PTA (Phosphotungstic Acid) and PT (Phosphotungstate) stains of ultra thin sections after postfixation with Potassium Permanganate:
Potassium permanganate has been used as a post-fixation in a manner similar to secondary fixation with osmium tetroxide. Preliminary results indicate that acidic PTA may be useful as a stain with this material, but that the results will not be as spectacular as with osmium post-fixed material.

3. Studies with PTA as a "Stain":
Experiments to determine the feasibility of staining ultra-thin sections of post-osmium fixed material with PTA after treatment with NaOH-alcohol (a process used with some light microscopic procedures) rather than oxone are in progress. Also, the practicality of staining ultra-thin sections of materials embedded in plastics other than Epon or Vestopal (i.e. Spurr's law viscosity medium and Araldite) with PTA is being studied. Studies are being carried out on the appearance of cortical tissue "fixed" in calcium containing fixatives.

ROCHELLE MEDICI and SUZANNE BAWIN

Effects of Modulated VHF Field on Brain and Behavior:
Instrumentation. We have completed instrumentation of a specially designed screened room to allow EEG recording of animals exposed to modulated VHF fields. Power supply, transmitter and function generator are located outside the room. The power supply unit was designed to provide modulated high voltage to the transmitter. The function generator provides sine waves of adjustable frequencies for the amplitude modulation of the signals. The unbalanced line from the transmitter is coupled to two large (4000cm^2) aluminum plates by means of a "balun". Reflections in transmitter cable are minimized by a "delta" impedance matching device. Plates configuration and position insure a uniform capacitance per unit length, the field intensity is estimated to be less than 2 mw/cm^2, percentage of modulation is above 90. All lines are brought into and out

of the room through low pass filters of the "feed through" types (attenuation up to 120 db at 147 MHz).

2. Experiment:
As a first step in these experiments, we simply observed the animals behavior and brain wave patterns during short exposure to the VHF fields. The animals were restrained within two large metal plates, and modulation frequencies were varied within 0-32 Hz. Observation of the animals suggested that very slow modulation frequencies (0-5 Hz) enhanced somnolence and higher modulation frequencies enhanced wakefulness. An observable effect on the EEG was not discernable until after approximately three minutes of exposure. As a result of these observations we structured a short, simple experiment in order to study more systematically the effect of the modulations. The animals used in this experiment were submitted to random presentations of four conditions in 10 minute epochs. (Continuous wave exposure, fields amplitude modulated at 3 and 16 Hz and a field off condition). Progressive invasion of the EEG by slow waves and concurrent behavioral quietness were repeatedly observed with the 3 Hz modulation. Low amplitude, high frequency EEG, associated with stages of wakefulness and paradoxical sleep were predominant under the 16 Hz modulation exposures. No effect could be detected with unmodulated field exposures.

A more complex experiment was then assigned to assess modulated VHF field effects on specific brain activities. In this experiment EEG patterns from a variety of subcortical and cortical structures in a given cat were surveyed. An easily discriminated pattern in a particular structure was then selected and subjected to an operant reinforcement technique, (shock avoidance). As a result of this conditioning it was possible to obtain very specific localized rhythms, with a registerable rate of occurrance (in this case every 30 seconds).

Once the animal had been well conditioned (to a 70% level or better) he was extinguished to his original operant level. In the second phase of this experiment, the cats were reconditioned to a high level and the modulated VHF fields were introduced as a continuous background stimulation during overtraining. Frequency of the modulation was set at the center frequency of the conditioned EEG rhythm. The animals were then put on an extinctive schedule during which the VHF exposure was continued. Control cats were subjected to precisely the same kind of reconditioning and extinction, without any exposure to the VHF field. These experiments revealed a profound effect of the field exposure. Extinction trials persisted for as long as two months. Control animals by way of contrast, extinguished within 10 days. The specificity of the field effect on a particular brain rhythm in a particular structure, the gradual onset of the field effect, the fact that the conditioned patterns occurred in short bursts, only at fixed time intervals, and the eventual return to operant level in the extinction schedule, once again is strongly in favor of a genuine biological transduction.

We are continuing these experiments and a variety of related ones in an effort to better understand mechanisms of interaction and to replicate and extend the results at the level of behavior and neurophysiology.