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IMPLANTATION OF MULTILEAD ELECTRODE ASSEMBLIES

AND

RADIO STIMULATION OF THE BRAIN IN CHIMPANZEES

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José M. R. Delgado, M. D.

et al.

Yale University

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6571st Aeromedical Research Laboratory
Aerospace Medical Division
Air Force Systems Command
Holloman Air Force Base, New Mexico

Animals used in this study were handled in accordance with the "Guide for Laboratory Animal Facilities and Care" prepared by the National Academy of Sciences - National Research Council and in accordance with the Secretary of Agriculture Standards in "Laboratory Animal Welfare" (Federal Register, Vol 32, No. 37, February 24, 1967).

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FOREWORD

This study was conducted simultaneously at the Department of Psychiatry, Yale University School of Medicine New Haven, Connecticut, and the 6571st Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico, during the period of 1 November 1967 to 1 June 1968, under Contract No. F29600-67-C-0058, Project 6892.

Co-authors of this report are Ronald J. Bradley, Victor S. Johnston and Gerhard Weiss of Yale University and Jan D. Wallace, Captain, USAF, MC of the 6571st Aeromedical Research Laboratory.

This technical report has been reviewed and is approved for publication.

Robert G. McIver

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Commander

ABSTRACT

This report describes the technology developed for intracerebral implantation of multiple electrodes in the chimpanzee and subsequent multichannel radio stimulation and telemetric recording of brain activity. In conjunction with this study a photographic-histological technique has been evolved for serial analysis during stereotaxic sectioning of the brain.

INTRODUCTION

Permanent implantation of intracerebral electrodes in animals provides the opportunity to apply stimulations and make recordings in fully awake subjects while they engage in spontaneous activities or perform instrumental responses, and allows investigation of the neurophysiological mechanisms of individual and social behavior. Stereotaxic techniques are generally based on the instrument developed by Horsley and Clarke in 1908 (1) which is widely used in many laboratories, and several reviews of stereotaxis are available (2, 3, 4, 5, 6). For theoretical considerations, practical details, and bibliography about electrodes, surgical techniques, electrical stimulation and recording of the brain, a previous publication may be consulted (4).

Important information about brain functions in awake animals has already been obtained in rats, cats, and monkeys, while only a very limited amount of experimentation has been performed in chimpanzees (7, 8, 9). Because of its closer proximity to man in the evolutionary scale, its extensive and complex behavioral repertoire, and its superior intelligence in comparison with lower species, the chimpanzee is of exceptional inte st as the subject of investigations of the cerebral mechanisms of behavior. At the same time, the strength of these animals, their manual skill, and peculiar habits all pose greater technical difficulties.

The purpose of this report is to describe the technology developed and the experience gained in the study of a group of chimpanzees with implanted electrodes. The research had the following characteristics: (a) Intracerebral implantation of 60-100 electrodes in each animal for detailed mapping and functional analysis of many cerebral structures; (b) miniaturization of leads and connectors in order to increase the number of contacts while minimizing trauma and diminishing cosmetic interference; (c) provision for anchorage points in the skull for the attachment of instrumentation; (d) surgical and instrumental simplicity; (e) long-term reliability of implants; (f) provision for multichannel radio stimulation and telemetric recording of brain activity; (g) development of photographic-histological technique for serial analysis during stereotaxic sectioning of the brain.

METHODS

A. Subjects

Five chimpanzees with no previous behavioral training were used in this study, and information about them is presented in the following table.

The first two animals, No. 687 and No. 688, were initially with the group housed at Yale University in New Haven, Connecticut. After implantation of electrodes and testing for several months, they passed through a required quarantine period and joined the other three chimpanzees at the colony of the 6571st Aeromedical Research Laboratory in Holloman Air Force Base.

SUBJECT DATA

Animal Number	Sex	Weight (lbs)	Weight (kg)	Implantation Date	Number of Leads
687	M	39 1/2	17.02	13 Nov 67	100
688	M	36	16.34	1 Feb 68	100 plus instrument box
654	M	46	20.88	14 Feb 68	80 plus instrument box
245	M	50 1/2	22.93	3 Apr 68	60
655	M	55	24.97	4 Apr 68	60

B. Apparatus

1. Multilead electrode assemblies: Introduction of a wire into the depth of the brain is always a traumatic process which produces hemorrhage and destruction of neurons, but fortunately the functional consequences are usually negligible. The use of assemblies of electrodes with tips exposed at different points along the same shaft has the advantage of providing multiple coverage of several cerebral structures along the implantation tract with approximately the same effort and trauma accompanying implantation of a single lead. In this way, a far greater amount of information may be obtained with a minimum of cerebral disturbance.

Electrode construction, number of contacts, their distribution on the same or separate shafts, and other details are part of the experimental design for each animal. The following description details construction of the type of assembly implanted in chimpanzee No. 687 (Fig. 1): A length of stainless steel wire 0.12 mm in diameter, insulated with four coats of Teflon (Hitemp Wires, Mineola, N. Y.), was straightened between two hemostats and cut in lengths of 144, 135, 130, 125, and 120 mm. The insulation was scraped from each wire for 1 mm at one end and 4 mm at the other, and the longest wire was bent at the tip for 2 mm to form a hook. The five wires were cemented together with Plexiglas dissolved in dichloroethylene, spacing the 1 mm bare tips 5 mm apart. The 4 mm bare ends were soldered in identifiable order to pins 1-5 of a subminiature 20 pin Cannon socket. Three more assemblies of five contacts each were prepared in a similar manner and soldered successively to pins 6-10, 11-15, and 16-20 of the same socket. All soldered joints were covered, insulated, and waterproofed with epoxy cement. The multilead electrode assembly was then tested with an ohmeter to check location of contacts and insulation of leads. In this way, we had four shafts which could be implanted in four different brain tracts, connected to a single socket with a total of 20 leads. A further refinement consisted in using micropin Cannon sockets which are smaller and have a greater concentration of contacts in less space.

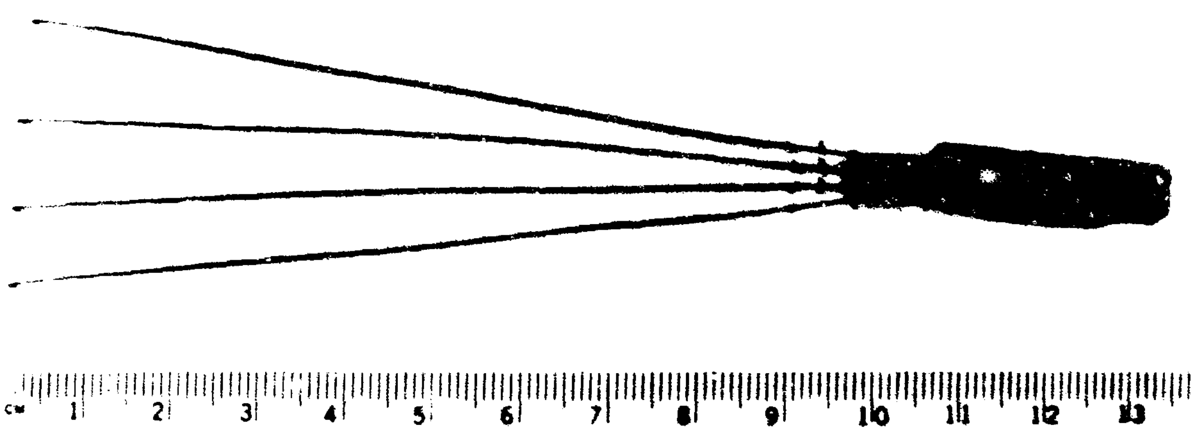

Figure 1. Multilead electrode assemblies formed by 20 contacts arranged in four shafts. The hook at the tip of each shaft is caught by 27-gauge stainless steel tubing during stereotaxic introduction into the brain. The leads are stainless steel wire covered by Teflon. The larger hole at the right of the socket has a threaded metallic receptacle for anchorage of instrumentation, as shown in chimpanzee No. 687 in Figure 2.

Figure 2. Chimpanzee No. 687 on the left and No. 688 on the right. Each animal has 100 electrodes implanted in the brain covering different areas. Instrumentation is contained in a detachable box mounted on top of the head sockets in Animal No. 687, and in No. 688, the instrumentation is inside a box which is permanently attached to the skull behind the head sockets.

2. Stereotaxic instrument: The instrument constructed by David Kopf (Tujunga, California) for stereotaxic surgery in the chimpanzee was used and was found accurate and satisfactory. Stereotaxic coordinates for implantation of electrodes were calculated from the atlas of DeLucchi, et al. (10).

3. Anchorage of instrumentation to the skull: Instrumentation may be carried by the animal on a small harness or on a collar around the neck with connecting leads attached to the sockets implanted in the head. This procedure is usually well tolerated by monkeys and chimpanzees, but in some cases the animals have pulled and broken the connecting leads, and to avoid this problem two procedures were devised:

The first one was rather simple, consisting of cementing to the lateral holes of the sockets an internally threaded metallic tubing 3 mm in diameter (Fig. 1). After implantation of the sockets in the skull, the metallic tubings provided a very solid anchorage for the attachment of instrumentation, as shown in Figure 2.

The other procedure was to implant a 48 by 32 by 18 mm Teflon box directly into the skull, behind the electrode sockets, as shown in Figure 2. This procedure was surgically more complex than the first method and entailed greater risk of infection, but there was no problem in either of the two chimpanzees prepared in this way, and the method is considered highly satisfactory.

4. Radio stimulation of the brain: A large number of instruments have been described in the literature for stimulation of the brain by remote control, but as stated in previous reviews (4, 11), the paucity of published results is an indicator of technical difficulties encountered in the use of these instruments. A problem common to the majority of these devices is that the intensity of brain stimulation is related to the intensity of the received radio signal which depends on the orientation of the receiving antenna, the distance from the transmitter, the grounding of the animal, the reflection of the waves, and the shielding of the receiver by obstacles or by other animals. These problems have been eliminated in the system developed for our experiments which consists of two instruments: (a) the RF transmitter, which measures 30 by 25 by 15 cm and includes the necessary electronics for controlling the repetition rate, duration, and amplitude (intensity) of the stimulating pulse. Panel controls allow variation of the repetition rate in steps between single pulses and 200 Hz and pulse duration between 0.1 and 1.5 msec. The intensity is controlled by varying the frequency of three subcarrier oscillators which operate in the 100-500 K Hz range. A 100 MHz oscillator is activated by the train of pulses originated in the subcarrier oscillators, and its duration is controlled by a switch which determines the stimulation time. The bursts of 100 MHz RF energy are received by the second part of the system,

(b) the receiver stimulator, which is carried by the subject (Fig. 2), measures 37 by 30 by 14 mm and weighs 20 g, including a 7 v Mercury battery. The instrument has a solid state circuitry encapsulated in epoxy resin to make it waterproof and animal proof. The antenna is a flat piece of metal which forms part of the box. The RF received signal is detected and the resulting subcarrier frequency is demodulated into an amplitude which controls the current intensity of the stimulation pulse. As intensity is related to the frequency of the subcarriers, it is independent of changes of strength in the received signal, making the instrument highly reliable. The output is constant current and therefore independent of wide change in biological impedance. Under normal conditions, the battery life is about one week, and operating distance is 100 feet. Stimulation parameters may be controlled in three separate channels.

5. EEG Telemetry: A miniature FM-FM amplifier-transmitter combination and a telemetry receiver are used for this purpose. (a) the transmitting circuitry, carried by the subject, consists of an EEG amplifier with a gain of 100, input impedance of 2 MΩ, frequency response from 2-200 Hz, and a voltage controlled oscillator (VCO) for each channel. The VCO operates in one of the frequency bands assigned for subcarrier oscillators by the IRIG standards. In these studies, a three-channel system was used which operated on IRIG channels 11, 13, and 14. The outputs of all three subcarrier oscillators were summed and connected to the single RF transmitter module. The miniaturized RF transmitter operates at 216 MHz and its range is 50 to 200 feet.depending on the environment. The size of the three-channel unit, including the battery, is 4.5 by 4.5 by 1.5 cm, and it weighs 50 g. The signals from the depth electrodes are received by the amplifier. The output signal of the amplifier controls the frequency of the subcarrier oscillator, and the oscillator output in turn controls the frequency of the transmitter; (b) after amplification of the received signal from the transmitter has been demodulated, the composite subcarrier signals are connected to the inputs of the three discriminators which then separate and demodulate their respective subcarriers to obtain the telemetered analog information. In the instrumentation used in this instance, a 100 μv signal at the EEG amplifier resulted in a one v output from the corresponding discriminator in the receiver. Figure 3 shows a sample of EEG recorded from animal No. 688 by this method.

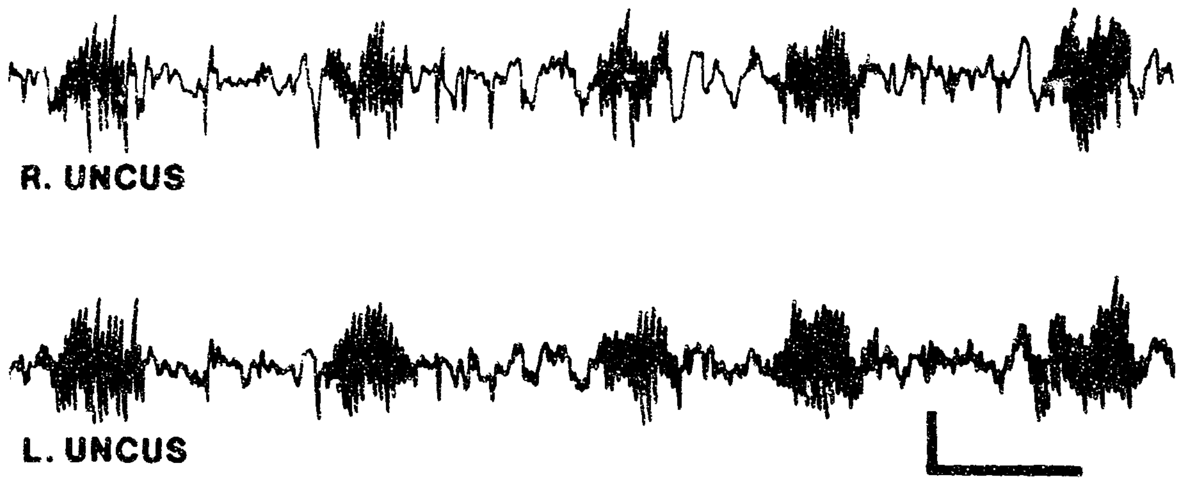

Figure 3. Telemetered EEG from Left and Right Uncus of Animal No. 688. One second and 50 μv.

6. Stimoceiver: Multichannel stimulation and telemetry of the brain have been integrated into one instrument called "Stimoceiver" (stimulator and EEG receiver) which weighs 70 g and has already been used in monkeys, chimpanzees, and human patients with behavioral disturbances. A description of the block diagram of the stimoceiver is presented in the appendix of this paper. Details about electronic designs and circuitry will appear in another report (See also 12, 13).

7. Additional equipment: Time-lapse photography, analysis of social behavior, instrumental responses, electroencephalographic recording of the brain activity, tape recording of vocalizations, and studies of the effects of brain stimulation have been conducted in this group of chimpanzees and the results will be described in future communications for which the present report is the methodological basis.

C. Procedure

1. Surgery for implantation: The animals were operated on under intravenous Diabutal anesthesia (30 mg/kg). The head was shaved

and fixed to the Horsley-Clarke instrument. Under aseptic precautions, the animal was draped and surgery started with a 6 cm midline incision of the scalp, reflecting the skin on both sides, and scraping the periosteum. The points selected to implantation were marked and a 2 cm in diameter trephine hole was made. The dura was opened by a semicircular section and the surface of the brain inspected to locate sizeable blood vessels on the cortex. Using the micromanipulator of the instrument, a 27-gauge needle was used as an introducer, catching the hook at the tip of each electrode shaft to guide it down to a precalculated depth. The base of the shaft was tied to a vitallium screw anchored at the edge of the bone hole. Then the introducer was withdrawn and the process was repeated for every electrode shaft The dura was sutured and covered with gelatine foam. The bone defect was filled with dental cement Dura Lay (liquid and white powder #65, Reliance, Dental Mfg. Co., Chicago, Ill.). The terminal sockets of the electrodes were anchored as a single block to the skull with the aid of eight vitallium screws, stainless steel wire and dental cement. The skin was closed around the implanted block.

In the two chimpanzees with instrument boxes, the scalp opening was wider and the base of the box had a 1.5 mm edge with small holes to pass through stainless steel wire to be anchored to vitallium screws attached to the skull. All spaces were then filled with dental cement.

2. Serial photohistology of the brain. In the stereotaxic atlas of the chimpanzee published by DeLucchi *et. al.* (10), it is mentioned that fixation and celloidin inclusion for sectioning resulted in a shrinkage of the brain of 26 per cent. This fact introduces a considerable distortion in the histological analysis. In addition, inclusion in celloidin is a rather time consuming process. Some of these drawbacks are avoided by using the following procedure which was developed in our laboratory for monkeys and may also be applied to chimpanzees: The animal is anesthetized and perfused through the heart with saline and 10 per cent formaline. The skull is opened with a saw and the head placed in the stereotaxic instrument. The brain is then cut through coronal planes, 0, 10, 20, and 30 by means of a knife driven between two metallic plates positioned in the corresponding plane by the stereotaxic instrument. The brain is extraced from the skull and immersed in formaline. Each 10 mm block is then placed on the object plate of a Jung Tetrander Microtome and immediately frozen by means of a standard histofreeze unit (Forma Scientific

Company, Marietta, Ohio). An Exakta camera is mounted above the microtome and loaded with Kodak High Contrast copy film. Pictures are taken with a ring flash unit attached to the lens of the camera while the brain is being sectioned at 50 μ (Fig. 4). As a routine we take one picture every five sections, totaling four pictures every mm of brain, and 40 pictures per 10 mm block. These photographs give adequate anatomical details for localization of contacts and also permit accurate reconstruction of the whole brain. Storage of the films is very economic in space and weight.

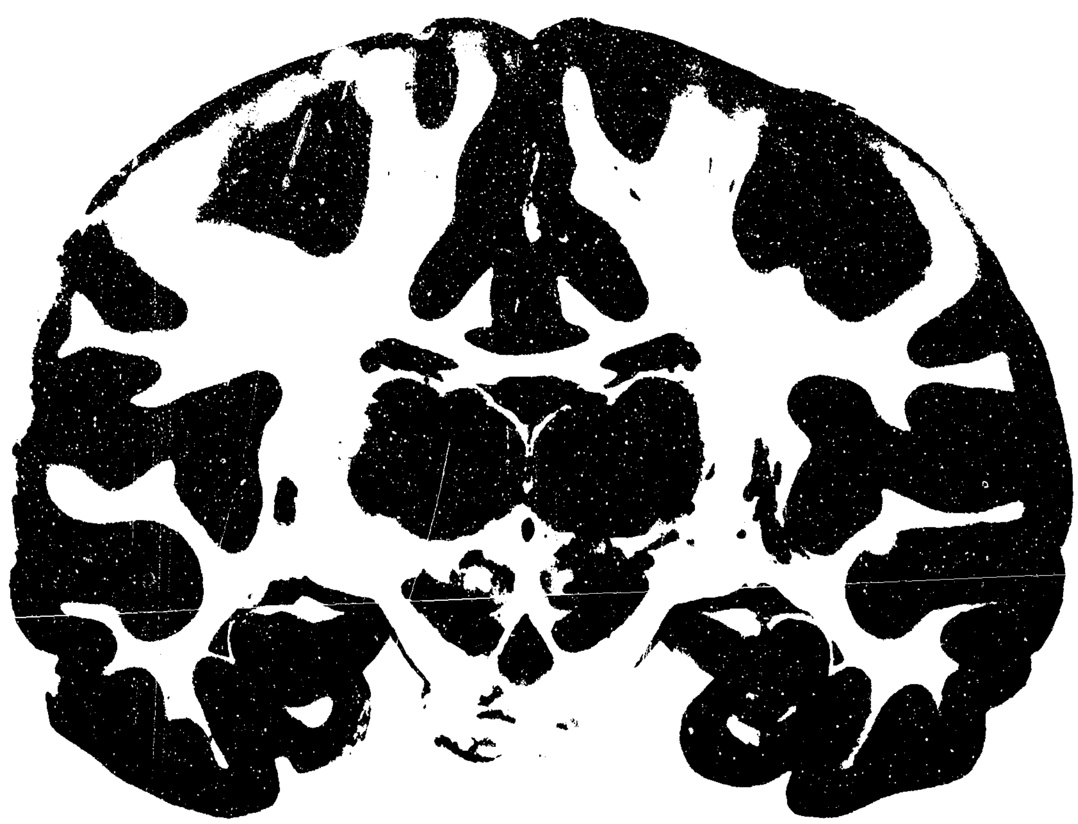

Figure 4. Unretouched photograph obtained during stereotaxic sectioning of the frozen brain. The tip of one electrode is visible in the right hippocampus. (The chimpanzee belongs to a different series because the animals in the present study are all alive).

RESULTS

The five chimpanzees have tolerated very well the large number of electrodes implanted in their brains. No functional deficits were discovered and the animals have been in excellent health to date. The anchorage of the terminal sockets to the skull has proved solid enough to withstand tampering by the animals and occasional head banging, and all electrodes have remained functional.

Initial experiments have been conducted in the restraining chair where recordings, stimulations, and instrumental responses were performed. In this situation, the chimpanzees were connected by long leads to the instrumentation with the advantages that the passage of current through the brain could be monitored by the oscilloscope, the socket connections were easily changed, and the animal was readily accessible. The main disadvantages were the behavioral distortion and limitation imposed by restraint and the restlessness of some chimpanzees, especially if the experiments lasted for too long. These handicaps were avoided by using stimoceivers which permitted the establishment of a two-way radio link for stimulation and recording while the animals enjoyed complete behavioral freedom.

One example of the inhibitory effects of caudate stimulation is presented in Figure 5. Chimpanzee No. 687 was trained to press a lever for food reward, and he was highly motivated for prolonged periods at the usual schedule of one reinforcement every 10 responses. This instrumental response was inhibited for the duration of radio stimulation of the head of the caudate nucleus, as demonstrated in Figure 5. As soon as stimulation started, the animal lowered his hands, bent his head slowly forward and down, lost all interest in the lever, and looked to the left with a facial expression of diminished awareness. At the end of stimulation, normal expression was regained in a few seconds, and lever pressing was continued at a similar speed as before stimulation. These excitations also had pacifying effects, making the animal more docile, less aggressive, and hyporeactive toward sensory stimulation. He could, however, voluntarily override these inhibitory effects if he was strongly motivated by fear or hunger. The stimulation parameters were cathodal, monopolar excitation, 100 Hz 0.5 msec pulse duration, 1.2 ma, lasting for 5 seconds. The results of stimulations and recordings of the brain in the five chimpanzees will be presented in detail in a forthcoming report.

Figure 5. (Top) Chimpanzee No. 687 pressing a lever during positive reinforcement for food. (Bottom) Inhibition of instrumental response during radio stimulation of the head of the caudate nucleus.

DISCUSSION

Stereotaxic methodology has the inherent handicaps of anatomical and physiological variability of the brain. Craneo-cerebral correlations are not constant, and a fact often overlooked is that functional differentiation of neuronal structures is to a great extent related to individual experience which therefore introduces unpredictable elements of variability. Functional exploration is essential for the improvement of stereotaxic surgery (14), and as an alternative we may compensate for variability by multiplicity of implanted leads. For this reason it was important to evaluate the practicality of massive implantations. Our study demonstrates the chimpanzees tolerate well the introduction of 100 contacts in the brain. Further miniaturization using thinner wires and smaller sockets with a higher density of contacts, such as the Cannon micropins, will make it possible to implant several hundred contacts in a single brain. A detailed functional mapping of determined structures will thus be possible and will clarify vagaries of the available literature; e.g., about the representation in the basolateral and corticomedial amygdala. The demonstrated stability of implants and evoked effects allows the planning of long-term studies.

Development of stimoceivers and the practicality of carrying them in the "instrument boxes" implanted into the skull provides a convenient procedure for a two-way radio communication with the brain without physical restriction of the subject's movements, paving the way for the investigation of neurophysiological activities during individual and social behavior in completely free subjects. Therapeutical application of the stimoceivers has already been successfully tested in four patients with intracerebral electrodes, suffering from behavioral disorders (13). The possibility to monitor continuously the electrical activity of the brain permitted the establishment of correlations between neuronal discharges and behavioral responses, demonstrating the usefulness of the described methodology in both scientific and medical applications.

SUMMARY

Technology has been developed for stereotaxic implantation into the brains of chimpanzees of large numbers of electrodes, and for the attachment of instrumentation to their heads in order to establish a multichannel two-way communication with intracerebral structures in completely unrestrained animals.

The characteristics of this technology are as follows: (a) Implantation of up to 100 electrodes; (b) miniaturization of leads and connectors; (c) provision for anchorage of instruments to the skull; (d) operational simplicity and comfort for the animals; (e) development of stimoceivers, allowing three channels of radio stimulation with individual control of pulse duration, frequency, and intensity, plus another three channels for EEG recording; (f) development of a photographic-histological procedure for serial analysis during stereotaxic sectioning of the brain.

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APPENDIX

Stimoceiver Block Diagram

The block diagram shows the four basic components of the stimoceiver:

a) The stationary transmitter is at the left.

b) The stationary receiver is at the right, and the small pack which is carried by the animal is shown at the center of the diagram with its two parts for

c) brain stimulation, and for

d) EEG recording, each one with three separate channels.

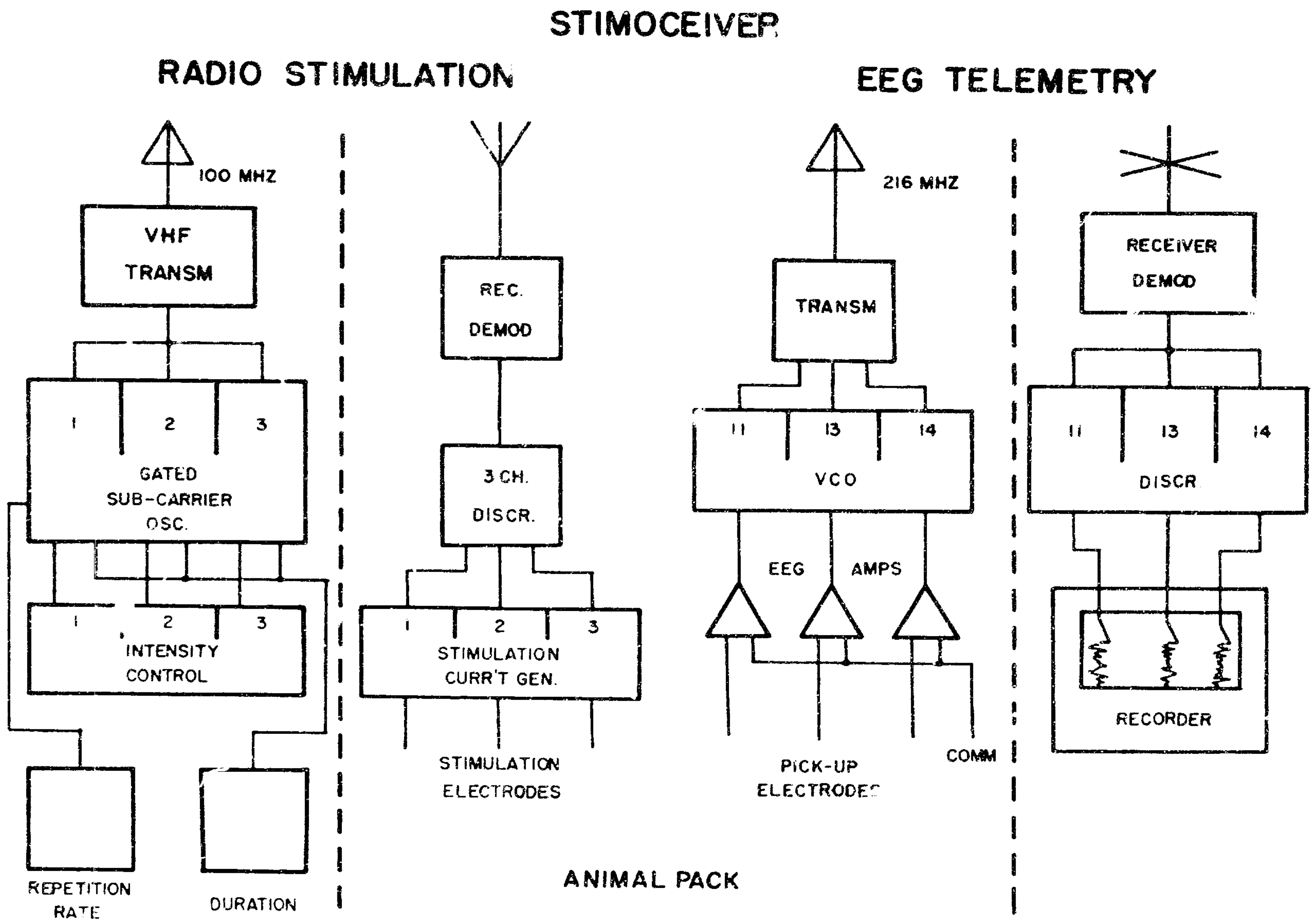
Radio Stimulation

The repetition rate of the stimulation pulses is controlled by a multivibrator whose frequency can be varied electrically. The pulses from this multivibrator operates the gating circuitry of the subcarrier oscillators in conjunction with the on-off switches for each channel and the duration control which is common to all three subcarrier oscillators. The intensity of the stimulation pulses is varied by varying the subcarrier frequency. The output from the gated subcarrier oscillators modulates the 100 MHz transmitter.

The transmitted 100 MHz signal is received and demodulated in the receiver carried by the animal. The demodulated signal is connected to the three channel discriminator where it is separated into channel and intensity information and activates the appropriate constant current generator to supply a current pulse of proper intensity and duration to the stimulation electrodes.

EEG Recording

The EEG telemetry part of the system consists of three differential EEG amplifiers which receive their signals from the pick-up electrodes implanted within the brain. The output of the amplifiers regulates the frequency of three voltage controlled oscillators (VCO) which operate on the designated IRIG frequencies. The VCO frequencies are added and modulate a VHF transmitter operating at 216 MHz.


STIMOCEIVER
RADIO STIMULATION
EEG TELEMETRY
100 MHZ
VHF TRANSM
1 2 3
GATED SUB-CARRIER OSC.
1 2 3
INTENSITY CONTROL
REPETITION RATE
DURATION
REC. DEMOD
3 CH. DISCR.
1 2 3
STIMULATION CURR'T GEN.
STIMULATION ELECTRODES
216 MHZ
TRANSM
11 13 14
VCO
EEG AMPS
PICK-UP ELECTRODES
COMM
RECEIVER DEMOD
11 13 14
DISCR
RECORDER
ANIMAL PACK

The signals from the transmitter are received and demodulated by a stationary receiver. The composite signal containing the frequencies of all three VCO is then separated into three individual channels and the analog information is retrieved from the subcarrier frequencies in the discriminators. The output of the discriminator may then be connected to a recorder.

The EEG telemetry system was developed by Drs. John P. Meehan and Roland D. Rader. (Multiple Channel Data Acquisition System for restrained and mobile subjects AF 04 (695) - 178 July 1965 Space System Division, Air Force Systems Command, Los Angeles 45, California). The three channel radio stimulator system and its integration with EEG telemetry was developed at our Yale Laboratory.

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13. ABSTRACT

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Unclassified

Security Classification