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OPERANT CONDITIONING OF AMYGDALA SPINDLING IN THE FREE CHIMPANZEE

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INTRODUCTION

It is known that bursts of spindles coincident with respiratory movements can be recorded from the olfactory bulb and amygdaloid nucleus^{1,2,13,17,20,21,23,28}.

These spindles are determined by the flow of air through the nostrils, as demonstrated by the fact that mechanical obstruction of the nose, local anesthesia of the nasal mucosa, or tracheotomy, results in disappearance of the spindles. The mechanism of propagation of this activity to the amygdala is not well understood. It could be related to olfactory functions, and it is somehow influenced by the state of arousal of the animal.

Gault and Coustan¹⁶ maintained that since the amygdala bursts do not persist in the absence of nasal air flow, the role of emotion could be explained by respiratory changes associated with arousal. Pagano²⁶ has revealed, however, that brain stem stimulation can produce an augmentation in olfactory bulb activity independent of nasal air flow. It seems that the passage of air through the nostrils is absolutely necessary for the presence of spindle activity, while its amplitude and its propagation to the amygdala depend on several factors, including emotional state and arousal.

One problem in the interpretation of the above mentioned experiments is that they were performed in animals under anesthesia, or restrained by the electrical wires necessary to establish connections between brain and instrumentation.

The research reported in this paper was a long term study of amygdala spindles in the free chimpanzee using telemetry to avoid behavioral disturbances in order to determine: (a) spontaneous occurrence during sleep and wakefulness; (b) behavioral correlations; (c) body movement correlations; (d) phase relation between right and left amygdala; and (e) effect of sensory stimuli.

In the second experimental phase we investigated operant conditioning of amygdala spindling. It is known that both classical and operant conditioning may induce changes in the discharge of single cells²⁵ and also in evoked potentials¹⁴, but in these experiments no related behavioral modifications were described. Wyr-

wicka and Sterman³⁴, working with awake, food deprived cats, demonstrated that the spontaneous occurrence of a 12–20 c/sec slow wave spindle in the sensory motor cortex was increased by its reinforcement with milk given to the animal, and this effect was accompanied by stereotyped posture and complete cessation of motility.

In our investigation, we used the spontaneous occurrence of amygdala spindles to trigger automatically electrical stimulation of another cerebral point located in the reticular formation which diminished further amygdala spindling. This experiment, in addition to testing operant conditioning, was intended to demonstrate the feasibility of establishing a long term, 2-way radio communication between our instruments and the brain of a free animal. The studies were conducted only in one chimpanzee, but they were repeated many times over a period of 1.5 years.

MATERIALS AND METHODS

Subject and electrodes implantation

The subject was a male juvenile chimpanzee named Paddy (age 6 years; weight 17.70 kg). On February 1st, 1968, with the animal under Diabulal anesthesia, 20 assemblies of 5 contacts each (a total of 100 electrodes) were implanted bilaterally in caudate nucleus, amygdala, and reticular formation*. The electrodes were made of stainless steel wire, insulated with teflon, with tips exposed for 1 mm and spaced at 5 mm intervals along the shaft. The procedures have been described in detail in previous publications^{6,11}. In addition, a teflon box measuring 48 mm × 39 mm × 18 mm was anchored with vitalium screws and dental cement to the animal's skull just behind the electrode sockets. This box provided housing for radio instrumentation.

The chimpanzee was trained to sit in a special chair¹⁸ where preliminary experiments were performed to record the spontaneous electrical activity of all points, and to apply electrical stimulations in order to evaluate motor and behavioral responses. The recordings showed a remarkable spindling activity in both amygdalas which was absent from all other areas studied, and we decided to study this phenomenon in detail. Four other chimpanzees were also implanted with a total of 60–100 electrodes each but no amygdala spindling was recorded, and only incidental information about them will be presented here.

Stimoceiver for remote controlled recording and stimulation

The integration in one instrument systems of multichannel radio-stimulation of the brain and depth EEG telemetric recording constitutes the Stimoceiver (*Stimulator and EEG receiver*) as described in detail elsewhere^{9,10}. The stimoceiver permits a 2-way radio communication between instrumentation and different areas of the brain in completely free animals. Pulse width, frequency duration, and intensity (constant current) of stimulation are controlled remotely.

* The stereotaxic atlas of the chimpanzee brain made by DeLucchi *et al.*¹² was used to calculate the implantation sites.

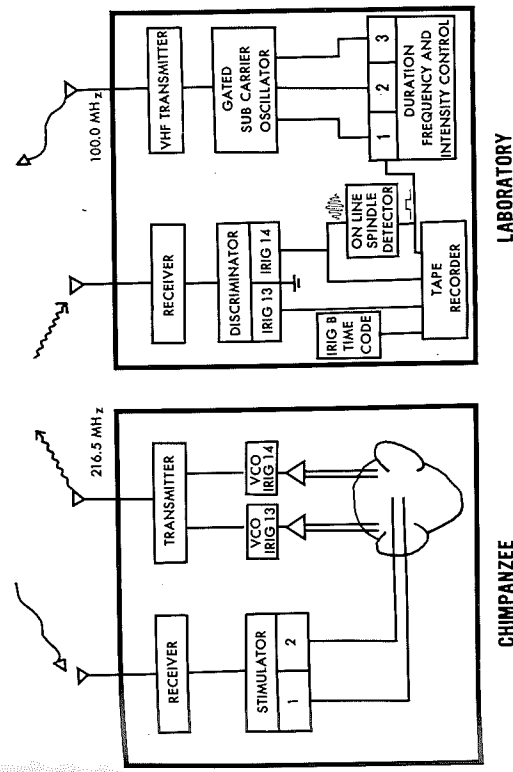


Fig. 1. Diagram of the experimental arrangement for operant conditioning. Brain waves sent by telemetry are received and their pattern recognized by the on-line spindle detector which triggers a stimulator sending radio impulses back to the brain.

Spindle detection and automatic contingent stimulation

The experimental arrangement, shown in Fig. 1, provided an artificial feedback between 2 cerebral structures by means of a radio link. The spontaneous electrical activity of right and left amygdalas was recorded bipolarly, being amplified, coded and transmitted by the FM section of the stimoceiver. The signals were then received, amplified, and demodulated in an adjacent room by means of a Nems-Clarke Receiver, Type R 1037F. The subcarrier information was fed into two Nems-Clarke discriminators, Model 67F, and the analog outputs were recorded on a magnetic tape Ampex FR 1200. At the same time the outputs from either discriminator were filtered to a band of 25–31 c/sec which was fed to a Donner Analog Computer, Model 3500, programmed to recognize the occurrence of spindles automatically and to produce a square wave output for the approximate duration of each spindle burst. These square waves were recorded in the tape along with the amygdala activity, counted electronically, and used to trigger the radio-stimulator. Stimulation signals were thus sent by FM radio to the animal, received by the corresponding electronics of the stimoceiver, and applied to one or both sides of the reticular formation of the chimpanzee.

In addition, unfiltered spontaneous electrical activity of both amygdalas was analyzed automatically by a Time-Data 100 special purpose digital computer in order to determine the power spectral density of different frequencies, from 0.5 up to 100 c/sec, through many hours of recording.

Sensory discrimination

In order to investigate the possible influence of visual and olfactory sensations

on amygdala spindles, the chimpanzee equipped with the stimoceiver, sat in a chair located in a chamber which was well ventilated, sound-deadened, and illuminated with a 100 W light. In addition, the chamber had a pellet dispenser, an odor dispenser, and provision for a flashing light. A tube near the head of the animal provided a continuous flow of laboratory air or of a selected odor produced by bubbling air through a suitable liquid. A system of tubes and valves enabled the experimenter to select odors without interrupting or changing the constant air flow. The chimpanzee was trained to put one hand at the delivery opening of the reinforcement mechanism to receive a food pellet.

At the end of a 30 sec presentation period of the sensory cue (odor or flashing light), the correct response of reaching for food was rewarded by a pellet. Failure to respond resulted in the food pellet being dropped to the floor, visible but out of reach. Square wave pulses marking 30 sec prior to stimulus presentation, onset of olfactory or visual stimulus, and delivery of reinforcement were recorded on magnetic tape simultaneously with the telemetered EEG activity of both amygdalas. This procedure enabled us to compute the spindle activity power by means of the TD-100 and to average its value for each daily session prior to, during, and immediately following presentation of visual and olfactory stimuli (see Fig. 5).

Telemetry of social mobility

Direct observation indicated a relation between spontaneous mobility of the chimpanzee and amygdala spindles. For its evaluation, the animal was equipped with the stimoceiver and also with a nylon belt carrying a small transmitter to detect body activity. This instrument consisted of a small stainless steel ball running free within the emitting coil of an FM transmitter⁵. The chimpanzee was completely free in the company of 3 other animals which were also equipped with mobility belts. The group was located on an artificial island formed by a subfield measuring 100 ft. in diameter, surrounded by a water-filled moat. This island was adjacent to, but isolated from the main chimpanzee colony maintained in the large consortium at Holloman AFB, N. M.³¹. The 4 experimental animals ate and slept inside of the brick consortium building and had free access to the artificial island where they spent most of the day. Receiving antennas and preamplifiers were mounted on a pole within the subfield and inside the building, permitting 24 h monitoring of the activity of each animal. Additional information about spontaneous behavior of the chimpanzees on the island was obtained by 16 mm time lapse color photography, taking one frame every 5 sec according to procedures described elsewhere⁷. Analyses of these data were then correlated with spindling activity.

RESULTS

Tolerance of electrodes and instrumentation

Our study demonstrated the practicality of long term implantation of large

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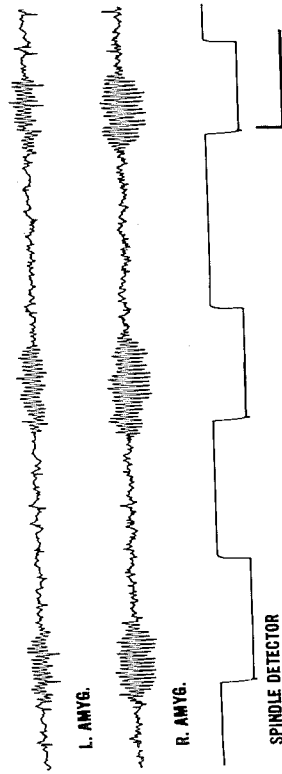


Fig. 2. Spontaneous activity from left and right amygdalas recorded by telemetry. The third channel shows the square wave output of the on-line spindle detector. These signals were used to trigger radio-stimulation. Calibration for Figs. 2 and 6: vertical line = 50 μ V; horizontal line = 1 sec.

numbers of electrodes within the brain of the chimpanzee. The 5 operated animals did not show behavioral disturbances and they have tolerated the cerebral electrodes to date (more than 2 years in one and more than 1.5 years in 4 other chimpanzees). During all this time, results of electrical stimulations were reliable, and spontaneous electrical activity was also constant and characteristic for different areas. This fact was substantiated most extensively in chimpanzee Paddy from whom more than 500 h of amygdala recordings were taken over 18 months of studies.

Anchorage of the instrument box to the skull was also well tolerated in the 2 chimpanzees in which it was used, and we recommend this procedure as preferable to the attachment of instrumentation to a collar, as in the other 3 chimpanzees.

Location of electrodes was calculated by the stereotaxic coordinates used and by the X-rays taken after implantation, but as the animals are still alive and in good health, histological confirmation is not yet available. One of the chimpanzees was sacrificed 15 months after implantation and the electrode tracts were about 1 mm in diameter with pathology similar to that described for the monkeys, used in other experiments⁴.

Spontaneous occurrence of amygdala spindles during sleep, wakefulness and mobility

In chimpanzee Paddy, spontaneous electrical activity of both amygdalas was characterized by flat voltage, below 20 μ V, interrupted by typical recurrent bursts which lasted for about 1 sec, with frequency around 28 c/sec, amplitude about 50 μ V and a general fusiform pattern as shown in Fig. 2. The inter-burst interval was about 2 sec, and this rhythm coincided with respiratory movements and changed with it. The burst amplitude was consistently higher in the right than in the left amygdala. This fact could be related to a difference in the precise location of the contacts, or to a functional asymmetry, but these possibilities were not explored further.

The power spectrum of frequencies between 0.5-60 c/sec was analyzed by the T-D-100 computer, revealing two distinct peaks, one at the low frequency band, and another at a higher frequency, around 28 c/sec, which corresponded to the olfactory bursts (Figs. 7 and 8). Filtering of this activity (Fig. 5) therefore gave a

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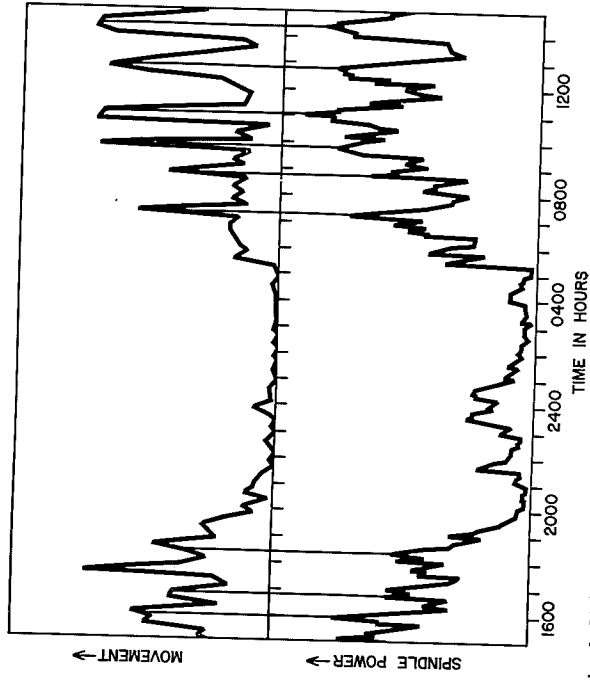


Fig. 3. Telemetric recording of animal's motility (upper line) and spindling activity of the amygdala (lower line). Observe the correspondence of peaks and the quiescence during the night. The animal was inside the consortium building in the company of the 3 other chimpanzees.

good indication of the magnitude of amygdala spindling. From all the 100 contacts, only the 2 pairs located bilaterally in the amygdala showed the above mentioned bursts. Other contacts, located 4 mm more posterior in the hippocampus, 5 mm more superior in the putamen and pallidum, or in more distant cerebral areas, failed to record olfactory bursts.

The persistence of amygdala spindling in Paddy was remarkable and the pattern, frequency, duration, and other characteristics of this activity were comparable throughout the many months of study. Its quantification by the automatic electronic counter revealed about 1.100 bursts/h occurring regularly throughout the day and interrupted from time to time by a few minutes of complete suppression. During this 'electrical silence' the animal was either sitting quietly or moving slowly about the home cage. Spindle activity was at its peak in amplitude and number when the chimpanzee was confronted by old friends, especially when Dr. Wallace and Dr. Bradley were present, and also at feeding time. Visits by strangers resulted in diminished and variable spindling.

To analyze temporal correlations, 10 periods of an uninterrupted 24 h were tape-recorded and the power spectrum of filtered frequencies 25–31 c/sec was then totalized and plotted against time. Direct observation of behavior had shown that the bursts of spindles decreased slowly during drowsiness, disappeared completely when the animal fell asleep, and reappeared spontaneously when the animal awoke. Automatic spectral analysis substantiated the previous observations, demonstrating the existence of a daily cycle in spindle activity which almost disappeared from about 8:00 p.m. to 6:00 a.m. The minor amount of spindles at night could not be correlated with any stage of sleep.

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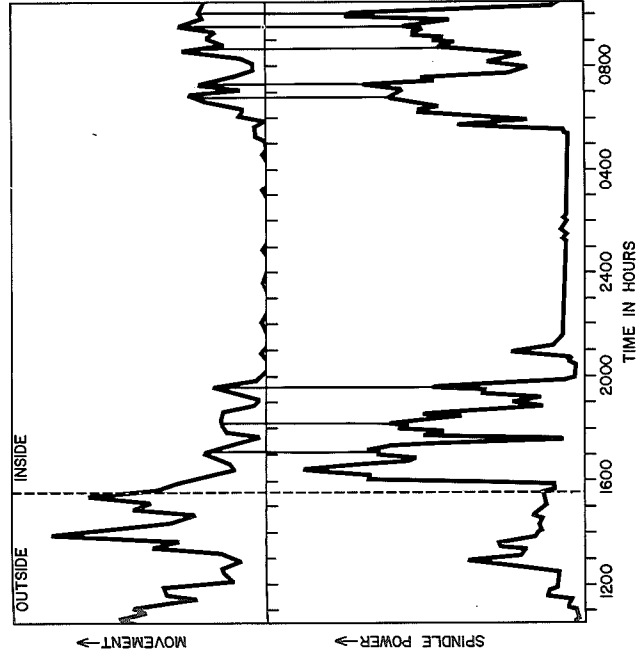


Fig. 4. Data similar to Fig. 3, but the animal spent some time free outside the building, on a small artificial island. During this time (from 10:00 to 15:30) the correlation between movement and spindling disappeared.

For a more objective study, telemetric recordings were made simultaneously of total body movements and spindling activity. In some of these experiments, Paddy was placed with 3 other chimpanzees in a large cage inside the consortium building, and representative data are shown in Fig. 3. At night power spindle and mobility were greatly reduced, while during the day both values increased considerably, with a highly significant correlation (Kendall's τ 0.83) between the peaks of both.

In other experiments the group was allowed to spend part of the day in the artificial island, outside the building. The data, presented in Fig. 4, confirmed the close correlation between body movements and amygdala spindling when the animal was awake and inside the building. This correlation was, however, lost when the animal was in the more normal ecological situation free on the artificial island. During the first hour, there was a considerable decrease in spindle power, in spite of the increased amount of motility, and later on the periods of greater mobility did not coincide with the peaks of amygdala spindling. Analysis of the time-lapse photographic recording of the group activity revealed that the periods of increased spindling coincided with reciprocal grooming between the chimpanzees.

Phase relation

Since spindle bursts almost always occurred simultaneously on both sides,

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phase relations could be computed easily by cross power spectral density and cross correlation techniques. Control of proper polarity was facilitated by a curious unidirectional spiking of constant polarity in both amygdalas which was taken as reference to orient the polarity of the spindle recordings in order to evaluate their shifts of phase.

It was observed that the average phase correlation of lower frequencies (0.5–20) were nearly in phase with an approximate 6° shift to right. In contrast the average phase over almost 20,000 spindle bursts was almost out of phase or approximately 160° to right. Single spindle bursts phase relationship was found to vary greatly over short periods from zero shift to plus or minus 180° shift in a seemingly random fashion. These facts indicate a considerable neurological independence of the bursts in each amygdala and also a variability in the transmission mechanisms.

Effects of sensory stimuli and local anesthesia

Presentation of orange odor, benzoin odor, or flashing a light induced a significant increase in spindle power. Further experiments showed that entrance of a person in the experimental room also increased the spindling activity of the chimpanzee. It was concluded, therefore, that the effect was nonspecific and probably related to increased arousal determined by the novelty of the stimulus.

Adaptation of the response was apparent, and after repeated presentation of these stimuli no changes in amygdala activity could be detected. One important exception was the orange odor which continued to increase the power of spindling significantly after more than 50 presentations, indicating specificity of the effect, and suggesting a role of amygdala spindling in olfaction. All increases in spindle power occurred simultaneously in both right and left amygdalas.

With the chimpanzee restrained in a chair, both sides of the nasal mucosa were anesthetized by applying a solution of 4% Xylocaine. About 15 min later spindles were absent and power spectral analysis of amygdala activity demonstrated an almost complete bilateral suppression of 28 c/sec waves (Fig. 8B). The animal was then tested for olfactory discrimination, as described in Methods, and showed a severe impairment in performance which was expected because with the nasal mucosa anesthetized, olfactory cues cannot be perceived. The residual amount of spindles and the minor success in a few olfactory discriminations were probably related to the difficulty in completely anesthetizing all the olfactory mucosa in a powerful and not very cooperative animal.

A strong ammonia odor provoked a defensive reaction in the animal, suppressing amygdala spindling for almost 30 min. When Paddy was ill with a bad cold for a few days the amygdala recording showed a considerable diminution of spindles.

Sensory discrimination

As described in Methods, the chimpanzee was trained to respond to orange odor and to flashing light for the reward of a food pellet. These olfactory and visual stimuli

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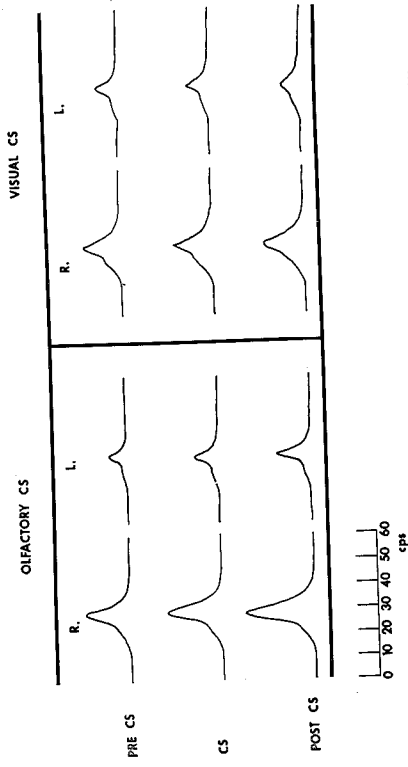


Fig. 5. Filtered activity (25–31 c/sec) corresponding to amygdala spindling, to compare olfactory and visual discrimination. Observe that olfactory testing increased the spindling power while visual sensation did not. For explanation see text.

were each presented 20 times, in random order, during the daily 6 h experimental session. Within 3 days the animal had learned both discriminations to the 100% level.

The average power of filtered activity (25–31 c/sec, which correspond to bursts of spindles) of right and left amygdalas, over 20 trials, is presented in Fig. 5 in order to compare the results of olfactory and visual discrimination. These averages correspond to the following three 30 sec periods: (a) immediately before sensory presentation (marked in the figure as Pre CS); (b) during sensory presentation (CS); and (c) immediately after sensory presentation (Post CS), when the chimpanzee was collecting his reward. Results demonstrated a significant increase in power spindle in both right and left amygdalas during olfactory trials. In contrast, there was no change in spindling during the experiments with visual cues, in spite of the fact that both situations were presented at random and that expectation of reward and general behavior of the animal was similar in both cases.

It was concluded therefore that conditioning caused an increased responsiveness in the power of amygdala spindling which was specific for the olfactory cue. This finding indicates the possible role of amygdala spindling in olfactory functions.

Operant conditioning of spindles

The chimpanzee was equipped with the stimocover to be in 2-way radio contact with instrumentation as shown in Fig. 1 and as explained in Methods. Each experiment had a total duration of 6 h, divided into 3 periods: The first 2 h were for control recording of amygdala activity and one example appears in Fig. 2. During the next 2 h the square wave output of the automatic spindle detector was activated by the telemetered activity of the right amygdala and each spindle triggered the radio-stimulation of the right reticular formation, which was therefore contingent upon and synchronous with spindling activity. The stimulation characteristics were cathodal, monopolar, 0.5 msec of pulse width, 100 c/sec, constant current intensity adjusted to 0.5 mA. When this stimulation was applied for the usual 1 sec duration of each

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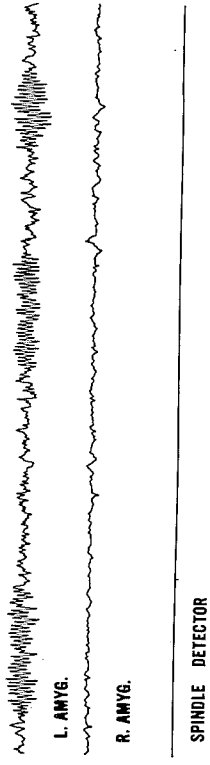


Fig. 6. After 6 days of automatic contingent reticular stimulation, the ipsilateral amygdala has suppressed its spindling activity.

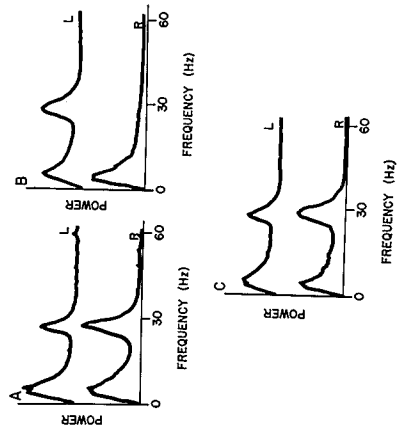


Fig. 7. Unfiltered averaged power spectral density of left (L) and right (R) amygdala. A, Control showing the typical 2 peaks at the low frequency band and at the 28 c/sec band. B, Six days after automatic contingent stimulation of the right reticular formation showing the block of spindling in the right amygdala. C, Control 14 days after the end of contingent reticular stimulations.

spindle, the observable effect was a minor facial grimace. Stronger intensities (1–1.5 mA) produced high pitched vocalization, restlessness and offensive-defensive manifestations, suggesting nociceptive sensations. These higher intensities, however, were not used for operant conditioning.

The third and last 2 h experimental period consisted of control recording without any stimulation.

Results showed that after 2 h of contingent reticular stimulation, the rate of amygdala spindling was reduced to about 50% of the control value. This effect persisted for the 2 h of the third period when reticular stimulation had been discontinued, and it was still present the next day during the first control period of recordings. The experiment was repeated daily, and after 6 days of contingent reticular stimulation the number of spindles were reduced to only about 6–8/h, representing a reduction to less than 1% of the normal spontaneous rate of spindling. One example of the bilateral amygdala activity at this time is demonstrated in Fig. 6. It is evident that the inhibition of spindling was exclusively ipsilateral, without any appreciable effect on the contralateral side.

Fig. 7 shows the unfiltered power spectrum of left and right amygdala activity

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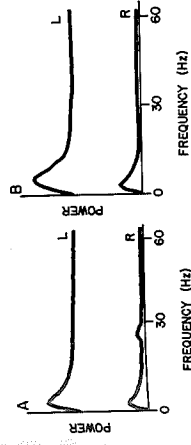


Fig. 8. Unfiltered averaged power spectral density of left (L) and right (R) amygdala. A, After 10 days of contingent bilateral reticular stimulation there was a suppression of amygdala spindling in both sides. B, Bilateral application of Xylocaine to nasal mucosa also blocks spindling in both sides.

during 1 h of recordings. Fig. 7A was obtained before starting the operant conditioning procedure and shows the usual two peaks at low and high frequencies, with coincidence in the spectrum of both sides, but with higher power in the right than in the left.

Fig. 7B represents the power spectrum after 6 days of contingent right reticular stimulation, demonstrating the total absence of the high frequency peak on the right side while the low frequency peak persists although with a slight shift to lower frequencies. In contrast, the activity of the left amygdala remained unmodified, and its high frequency peak is similar to that of controls.

This ipsilateral inhibition of amygdala spindling lasted for several days and then the number of spindles gradually increased returning to normal about 2 weeks after cessation of contingent reticular stimulation, as demonstrated in Fig. 7C.

Because the experimental results of sensory conditioning suggested an olfactory role of amygdala spindling, we were interested to know whether its lasting unilateral suppression had an effect on learned responses. For this purpose visual (flashing light), and olfactory (orange odor) discriminations were tested after 6 days of contingent reticular stimulation, when very little right-side spindling was present. Results showed that the chimpanzee was able to perform both discriminations with 100% of reliability. One additional experiment was then performed, anesthetizing the left nostril with Xylocaine, but even then, performance of sensory discrimination was not affected.

In the next series of experiments we tried to induce bilateral blocking of spindles. For this purpose the entire 6 h session was devoted to contingent stimulation. Computer detection of each burst of spindles in the left amygdala resulted in the triggering of two channels of radio-stimulation in order to excite simultaneously the right and left reticular formation. Within minutes after the start of this experiment, spindling in the right amygdala was blocked, demonstrating the residual effect of sessions performed several weeks earlier. Spindling in the left amygdala was also progressively suppressed and after 6 days of contingent bilateral stimulation, amygdala spindling disappeared in both sides.

The experiment was continued for 10 days, and at this time power spectral analysis of amygdala activity showed the persistence of the low frequencies peak and the absence of the peak of high frequencies in both amygdalas, as demonstrated in Fig. 8A.

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The chimpanzee was then tested for his ability to perform olfactory and visual discriminations which in spite of the complete absence of amygdala spindling were carried out without errors. There was, however, an important behavioral change: throughout the experiment the animal sat very quietly, performing the tasks without observing the food pellets being delivered and often without eating them. The lack of attention and motivation was evident, and the attitude of the chimpanzee was very different from his usual behavior during previous experiments. Outside of the testing situation the animal was also more placid than usual, without being excited by the presence of visitors or by the offering of appetizing food. This situation lasted for about 2 weeks after which the chimpanzee's behavior returned to normal and the spontaneous spindling of both amygdalas resumed their usual rate.

One important question required clarification: Was the amygdala contingency timing of the reticular stimulations specific and necessary for inhibition of amygdala spindles, or would the same amount of reticular stimulations without contingency also block amygdala spindling? To answer this question new experiments were performed with 2 h of control recording of amygdala activity, followed by 2 h of programmed stimulation of the right reticular formation. In this case the triggering of radio-stimulation was provided as usual by the square waves of the spindle detector, but instead of processing on-line information, we used the spontaneous amygdala activity tape recorded on a previous control day. Sequences, duration and total amount of reticular stimulation were therefore comparable in this and in previous experiments, the only difference being the lack of contingency. Results proved that in this situation there were no changes in the power or frequency of amygdala spindling in either side. It was concluded, therefore, that the contingency factor was essential for the suppression of amygdala spindling. During more than one year of intensive studies on Paddy, the operant conditioning of amygdala spindles has been repeated many times, and the experiment was presented as a live demonstration, with chimpanzee and equipment flown from Holloman AFB, N. M., at the Seventh International Congress of Electroencephalography and Clinical Neurophysiology held in San Diego, California, in September 1969.

DISCUSSION

The present paper has two main objectives: The first is the demonstration of the practicality and usefulness of recently developed technology for 2-way radio communication between laboratory instruments and the depth of the brain of free behaving subjects.

This methodology has hardly been employed in neuro-behavioral research and in our opinion it merits a far wider future use. Specially interesting is the principle demonstrated here that telemetered brain waves can be pattern recognized by a computer in order to trigger radio-stimulation of determined areas of the brain. In this way an artificial feedback between two cerebral structures can be established with the help of remote controlled instrumentation, paving the way for future 'on demand' stimulation of the central nervous system.

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The novelty of our approach has been merely to combine methods already in existence. Permanent intracerebral electrodes (10-16) have been implanted in chimpanzees²⁹. Telemetry of EEG is well known^{3,22}, pattern recognition of brain waves by computers has been described by several authors^{19,32}, and cerebral radio-stimulation has already had an initial acceptance in monkeys^{7,30}. Radio-stimulators and stimocivers were developed in our laboratory^{5,8}. The first clinical application of a 2-way radio link with the human brain was reported by Deldago *et al.*⁹ and the first 2-way communication between the brain of a free animal and a computer is described in the present paper. Neuro-behavioral research on the chimpanzee has an obvious interest because of the zoological proximity of this species to man, but the physical strength of these large apes makes it difficult to handle them and has deterred many scientists from working with anthropoids. Implantation of small instrumentation boxes in the skull may obviate many problems making possible long term radio-stimulation.

The second objective of this paper was to study the phenomenon of amygdala spindling in the chimpanzee, and although the experiments were performed only in one animal, testing was extensive and results were consistent. The animal is still alive and healthy and therefore we do not have histological confirmation of the location of electrodes as is usually the case in depth recordings in humans, but a strong argument in favor of accepting stereotaxic and X-ray data about localization of the recording contacts in the amygdala of our animal is that olfactory spindles are known to be highly specific of the amygdala.

The absence of spindle activity following anesthesia of the nasal mucosa demonstrates that nasal air flow is a necessary condition for the occurrence of amygdala spindles, in agreement with the results of MacLean and Delgado²³, Domino and Ueki¹³ and Gault and Leaton¹⁷. However, the nasal air flow is not by itself sufficient for amygdala spindling, because this activity disappeared at night when the animal was certainly breathing.

The state of arousal seemed to be an important factor for the propagation of olfactory bursts to the amygdala as it has been indicated by Freeman¹⁵, Lesse²¹, and Pagano and Gault²⁷. In the chimpanzee, according to Reite *et al.*²⁸, uncal spindling diminished during sleep, increasing, however, during paradoxical sleep in which 'dreaming' could be an emotive state. In our study, decreased arousal (sleep) coincided with absence of spindling, and increased arousal by sensory stimulation resulted in more power spindling. The relation between amygdala activity and arousal seemed to be reciprocal because when bilateral spindling was blocked by contingent reticular stimulation, the chimpanzee was less alert and less motivated. Very significant was also the correlation between spontaneous mobility and spindling power, although this correlation existed only when the animal was caged and could not be demonstrated when he was free in the artificial island. A possible explanation is that the richness of multisensory information in the island was certainly a different situation than the more restrained environment of a cage, and this fact emphasizes the importance of conducting neuro-behavioral studies in more normal ecological environments, and the danger of undue generalization of laboratory experiments.

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Amygdala spindling must somehow be related to olfactory functions, because in the sensory discrimination experiments, olfactory cues increased the power spectrum, while visual cues did not. Also, spindles increased swiftly after presentation of orange odor, and were more noticeable with the animal inside the consortium building where odors were relatively strong, than in the open space of the artificial island where the environment was ecologically more normal. However, the spindle bursts do not seem to represent olfactory information *per se* because we could not detect odor specificity in the frequency response of the bursts. The spindle activity could be related to a selective attention to olfactory cues, or perhaps to reverberations necessary for the initial consolidation of olfactory information.

Manipulation of brain activity by means of sensory or electrical stimulation has been described by Olds and Olds²⁵, who excited rewarding areas in rats in order to teach them to modify the rate of discharge of single units. Miller²⁴ also has described an increase of EEG voltage produced specifically by rewarding the change with electrical stimulation of pleasurable regions of the brain. In our experiments, the ipsilateral blocking of amygdala spindling induced by contingent reticular stimulation was an experimental fact repeated many times. Its explanation, however, is not clear. The noceptive properties of the stimulated reticular point could have a suppressor role, but it was remarkable that the precise timing of contingency was so essential and that the inhibitory effect was strictly ipsilateral, although when both sides of the reticular formation were stimulated, bilateral inhibition of spindles resulted.

In spite of the large number of electrical stimulations which could occur for up to 1100 sec in 1 h, there were no changes in local thresholds or in behavioral responses. This experimental reliability suggests the absence of histological lesions or functional alterations, in agreement with previous studies in monkeys stimulated chronically^{6,7}.

As demonstrated by Wetzel *et al.*³³, anodal stimulation with stainless steel electrodes produces histological lesions while cathodal stimulations, as used in our experiments, do not produce tissue effects. The absence of lesions in our results is also indicated by complete electroencephalographic and behavioral recovery of the chimpanzee, and by the fact that the same amount of reticular stimulation without contingency timing failed to produce any change in amygdala spindling.

SUMMARY

The aims of this paper are: (a) to show the practicality of 2-way radio communication between the brain of free animals and laboratory instrumentation; (b) to demonstrate the principle that telemetered brain waves can be pattern recognized by a computer in order to trigger contingent radio-stimulation of a determined area of the brain; and (c) to investigate amygdala spindling in the chimpanzee.

The experiments were performed on a juvenile male chimpanzee equipped with intracerebral electrodes, stimulator for multichannel EEG telemetry and brain radio-stimulation, plus mobility recording belt. Some of the studies were carried out

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with the animal completely free on a small artificial island in the company of 3 other chimpanzees.

Throughout 24 h, peaks of mobility corresponded with peaks of spindle power when the animal was inside a building, and not when he was on the artificial island. In this case spindling peaks correspond with periods of grooming.

Sensory excitation increased amygdala spindling. Visual sensation habituated while olfactory testing did not, suggesting a role of amygdala spindling in olfactory functions.

Automatic radio-stimulation of the reticular formation contingent on amygdala spindling suppressed ipsilateral spindling to less than 1% of control value. Bilateral reticular stimulation produced bilateral suppression of spindling accompanied by decreased motivation and behavioral placidity. The same amount of reticular stimulation lacking the contingency factor did not modify amygdala spindling.

REFERENCES

- 1 ADRIAN, E. D., Olfactory reactions in the brain of the hedgehog, *J. Physiol. (Lond.)*, 100 (1942) 459-473.
- 2 ADRIAN, E. D., The electrical activity of the mammalian olfactory bulb, *Electroenceph. clin. Neurophysiol.*, 2 (1950) 377-388.
- 3 BARWICK, R. E., AND FULLAGAR, P. J., A bibliography of radio telemetry in biological studies, *Proc. ecol. Soc. Aust.*, 2 (1967) 27.
- 4 DELGADO, J. M. R., Chronic implantation of intracerebral electrodes in animals. In D. E. SHEER (Ed.), *Electrical Stimulation of the Brain*, Univ. Texas Press, Austin, Texas, 1961, pp. 25-36.
- 5 DELGADO, J. M. R., Telemetry and telemetering of the brain. In L. SLATER (Ed.), *Bio-Telemetry*, Pergamon, New York, N.Y., 1963, pp. 231-249.
- 6 DELGADO, J. M. R., Electrodes for extracellular recording and stimulation. In N. L. NASTUK (Ed.), *Electrophysiological Methods, Vol. V, Part A: Physical Techniques in Biological Research*, Academic Press, New York, N.Y., 1964, pp. 88-143.
- 7 DELGADO, J. M. R., Free behavior and brain stimulation. In C. C. PFEIFFER AND J. R. SMYTHIES (Eds.), *International Review of Neurobiology, Vol. VI*, Academic Press, New York, N. Y., 1964, pp. 349-449.
- 8 DELGADO, J. M. R., Radio stimulation of the brain in primates and in man, *Anesth. Analg. Curr. Res.*, 48 (1969) 529-543.
- 9 DELGADO, J. M. R., MARK, V., SWEET, W., ERVIN, F., WEISS, G., BACH-Y-RITA, G., AND HAGIWARA, R., Intracerebral radio stimulation and recording in completely free patients, *J. nerv. ment. Dis.*, 147 (1968) 329-340.
- 10 DELGADO, J. M. R., AND MIR, D., Fragmental organization of emotional behavior in the monkey brain, *Ann. N.Y. Acad. Sci.*, 159 (1969) 731-751.
- 11 DELGADO, J. M. R., BRADLEY, R. J., JOHNSTON, V. S., WEISS, G., AND WALLACE, J. D., *Implantation of Multilead Electrode Assemblies and Radio Stimulation of the Brain in Chimpanzees*, Techn. Docum. Rept. ARL-TR-69-2, Holloman Air Force Base, N. M., 1969, pp. 1-19.
- 12 DELUCCHI, M. R., DENNIS, B. J., AND ADEY, W. R., *A Stereotaxic Atlas of the Chimpanzee Brain (Pan satyrus)*, Univ. Calif. Press, Berkeley, Calif., 1965, 78 pp.
- 13 DOMINO, E. F., AND Ueki, S., An analysis of the electrical burst phenomena in some rhinencephalic structures of the dog and monkey, *Electroenceph. clin. Neurophysiol.*, 12 (1960) 635-648.
- 14 FOX, S. S., AND RUDELL, A. P., Operant control of neural events. Formal and systematic approach to electrical coding of behavior in brain, *Science*, 162 (1968) 1299-1302.
- 15 FREEMAN, W. J., Correlation of electrical activity of prepyriform cortex and behavior in cats, *J. Neurophysiol.*, 23 (1960) 111-131.
- 16 GAULT, F. P., AND COUSTAN, D. R., Nasal air flow and rhinencephalic activity, *Electroenceph. clin. Neurophysiol.*, 18 (1965) 617-624.
- 17 GAULT, F. P., AND LEATON, R. N., Electrical activity of the olfactory system, *Electroenceph. clin. Neurophysiol.*, 15 (1963) 299-304.

Brain Research, 22 (1970) 347-362

- 18 GRUNZKE, M. E., *A Restraint Device for Behavioral Research with the Chimpanzee*, MDC-TDR 61-37, Holloman Air Force Base, N. M., 1961.
- 19 HANLEY, J., WALTER, D. O., RHODES, J. M., AND ADEY, W. R., Chimpanzee performance; computer analysis of electroencephalograms, *Nature (Lond.)*, 220 (1968) 879-881.
- 20 LAVIN, A., ALCOER-CUARÓN, C., AND HERNÁNDEZ-PEÓN, R., Centrifugal arousal in the olfactory bulb, *Science*, 129 (1959) 332-333.
- 21 LESSE, H., Rhinencephalic electrophysiological activity during 'emotional behavior' in cats, *Psychiat. Res. Rep. Amer. psychiat. Ass.*, 12 (1960) 224-237.
- 22 MACKAY, R. S., *Bio-Medical Telemetry*, Wiley, New York, 1968, 388 pp.
- 23 MACLEAN, P. D., AND DELGADO, J. M. R., Electrical and chemical stimulation of frontotemporal portion of limbic system in the waking animal, *Electroenceph. clin. Neurophysiol.*, 5 (1953) 91-100.
- 24 MILLER, N. E., Learning of visceral and glandular responses, *Science*, 163 (1969) 434-445.
- 25 OLDS, J., AND OLDS, M. E., Interference and learning in palaeocortical systems. In J. F. DELA-FRESNAYE (Ed.), *Brain Mechanisms and Learning*, Blackwell, Oxford, 1961, pp. 153-187.
- 26 PAGANO, R. R., *The Effects of Independent Manipulations of Control Stimulation and Nasal Air Flow on Induced Activity Recorded from Olfactory Structures*, unpublished Doctoral Dissertation, Yale University, New Haven, Conn., 1966, 95 pp.
- 27 PAGANO, R. R., AND GAULT, F. P., Amygdala activity: a central measure of arousal, *Electroenceph. clin. Neurophysiol.*, 17 (1964) 255-260.
- 28 REITE, M., STEPHENS, L., AND PEGRAM, G. V., Uncal spindling in the chimpanzee, *Brain Research*, 3 (1966/67) 392-395.
- 29 RHODES, J. M., REITE, M. R., BROWN, D., AND ADEY, W. R., Cortical-subcortical relationships of the chimpanzee during different phases of sleep. In M. Jouvet (Ed.), *Aspects Anatomio-Fonctionnels de la Physiologie du Sommeil*, C.N.R.S. Publ. No. 127, Paris, 1965, pp. 451-473.
- 30 ROBINSON, B. W., WARNER, H., AND ROSVOLD, H. E., A head-mounted remote-controlled brain stimulator for use on rhesus monkeys, *Electroenceph. clin. Neurophysiol.*, 17 (1964) 200-203.
- 31 VAN RIPER, D. C., FINEG, J., AND DAY, P. W., Development of a primate source, *Lab. Anim. Care*, 17 (1967) 472-478.
- 32 WALTER, D. O., AND ADEY, W. R., Spectral analysis of electroencephalograms during learning in the cat, before and after subthalamic lesions, *Exp. Neurol.*, 7 (1963) 481-503.
- 33 WETZEL, M. C., HOWELL, L. G., AND BEARIE, K. J., Experimental performance of steel and platinum electrodes with chronic monophasic stimulation of the brain, *J. Neurosurg.*, 31 (1969) 658-669.
- 34 WYRWICKA, W., AND STERMAN, M. B., Instrumental conditioning of sensorimotor cortex EEG spindles in the waking cat, *Physiol. Behav.*, 3 (1968) 703-707.

QUANTITATIVE ANALYSIS OF THE CEREBRAL CORTEX. II. A METHOD FOR ANALYSING BASAL DENDRITIC PLEXUSES

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INTRODUCTION

The relationship between neural structure and function is still rather obscure, partially due to our scanty knowledge of the histological structure and organization. This is particularly the case in a structure as complex as the cerebral cortex, where the basic problems involved in analysis of this structure are those of cellular heterogeneity, the organization of the intricate fiber networks and the meaning of the close apposition of neurons and glia.

Numerous histological parameters could be analyzed in search for correlations with functional states, but the most fruitful approach to the histological study of a grey substance such as the cerebral cortex seems to be a quantitative analysis of the constituents, nuclear and perikaryal volume, the patterns of the dendrites and axons, and the number and sites of the synaptic contacts.

In this paper of a series^{1,3} a method will be described that makes possible a rapid statistical analysis of the basal dendritic system of individual neurons. Using the model of Sholl^{1,2} for dendritic intersections through concentric spheres, the expected number of intersections through 2 concentric cylinders (*i.e.* circles in the eye-piece) will be calculated. With these data the parameters for the basal dendritic systems of the pyramids and the whole dendritic system of the stellate cells can be estimated.

In 7 neurons the model and the estimation method for the parameters will be tested.

MATERIALS AND METHODS

All analyses were done in preparations from 5 male, 4-month-old New Zealand rabbits.

The Golgi-Cox staining procedure (modification Van der Loos¹⁵) was used. The direction of sectioning was perpendicular to both the corpus callosum and the

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