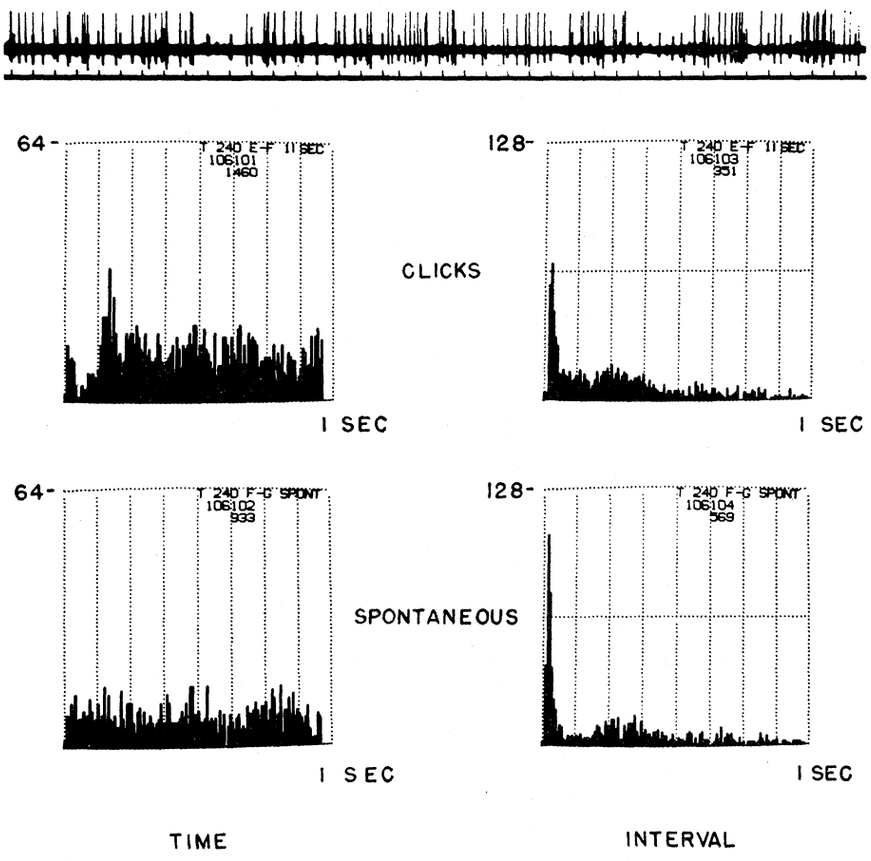

Fig. 1. Top trace, film strip of a cell's firing pattern. Lower trace, stimulus markers at 1 sec intervals. Below: time (left) and interval (right) histograms for 10 minutes of data from this unit. The stimulus condition is indicated at the upper right of each histogram (bin width, 8 msec).

Schmitt triggers, formed into standard TX-O computer pulses (by DEC digital test units), and fed directly into an available register of the computer. Detailed analog-digital conversion of the data waveforms is not used. The TX-O program senses the proper portion of the stimulus pattern, keeps internal time, and compiles the necessary histograms. The output of the processing program is a display of a histogram with various typewriter-controlled titling provisions; the display is photographed with a Polaroid camera. All relevant constants of the computation are available to the operator on toggle switches. The maximum resolution available in real processing time is 0.5 msec per bin. Better effective resolution can be obtained by playing back the data tape more slowly than it was recorded. If coarser resolution is used, the tape can be played back faster than it was recorded, and the processing time shortened.

The computer program accommodates tape-recorded experiments that involve repeated (cyclic) presentation of several different stimulus conditions. Each condition lasts for an arbitrary length of time and comprises an arbitrary number of repetitions of a basic stimulus. Such an experimental format allows meaningful comparison of the various stimulus conditions because data for all stimulus conditions are similarly affected by physiological changes of state of the preparation.

This processing system has been applied in a study of the firing patterns of single cells in the auditory cortex of lightly anesthetized cats (Nembutal). Single-neuron and slow-wave activity were observed by standard methods with 5 to 10 μ tungsten microelectrodes. In order to minimize the pulsatile motion of the brain the electrodes were moved in a closed oil-filled chamber threaded into the skull (*4*). Acoustic clicks were presented to the preparation in a repeating pattern of 15 seconds of stimulation followed by 15 seconds of silence. Both the electrical activity and the stimulus pattern were recorded with an FM tape system for later processing.

Results obtained for a cortical cell of a frequently encountered type are shown in Fig. 1. At the top is an ordinary strip-film presentation of the cell firing. The large pulses on the upper trace indicate firing of the neuron under study; the lower trace shows stimulus markers at 1 sec intervals. Below are time and interval histogram analyses of about 10 minutes of such data, for both the stimulated and spontaneous conditions. The time histograms show clearly that the cell's firings are depressed for approximately 150 msec after the delivery of the stimulus and then briefly enhanced. The interval histograms show less contrast between spontaneous and stimulated conditions. However, we note the distribution of interspike intervals is more uniform during stimulation. Since the cell is firing only about three times per second, it is extremely hard to "see" any of this behavior on the strip film.

Obvious extensions of these data-handling methods can lead to techniques that will be useful in investigating the complex relations between graded and discrete aspects of electrocortical function (*5*).

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Vivax-Type Malaria Parasite of Macaques Transmissible to Man

Abstract. Transmission of *Plasmodium cynomolgi bastianellii* from rhesus monkeys to two human subjects by *Anopheles freeborni* and the occurrence of attacks of malaria in two other laboratory workers not exposed to human malaria suggests the existence of an animal reservoir of infection complicating malaria control and eradication.

Plasmodium cynomolgi subspecies *bastianellii* was recently described by Garnham (*1*), who found distinct morphological and immunological differences between it and typical *P. cynomolgi*. The new subspecies was isolated from a *Macara irus* monkey from Malaya (*1*, *2*). Garnham sent this parasite to us for study, and we have done many experiments with it in rhesus

monkeys (*Macaca mulatta*). We have seen the differences described by Garnham between this subspecies and typical *cynomolgi*.

During a period in which very large scale inoculations of monkeys with sporozoites were being carried out, two of our staff members (D.E.E. and N.C.O.) developed illness with fever which, after a remittent period, proved to have a tertian periodicity. *Vivax*-type parasites with enlarged erythrocytes and Schüffner's stippling were found in both individuals. Smears were made on new slides, stained in clean dishes with new stain. Presumably the source of the malaria was mosquitoes infected with monkey malaria, as neither individual had had any recent contact with human malarias.

Five milliliters of blood from D.E.E. were injected intravenously into an uninfected rhesus monkey (W633). Eight days later parasites were present, and a typical *cynomolgi*-type infection ensued in which parasite densities of several hundred thousand per cubic millimeter were attained. Five milliliters of blood from N.C.O. were injected intravenously into another uninfected rhesus monkey (W673). Parasites were patent on the 10th day, and a normal infection ensued.

Two inmate volunteers were each given 10 ml of blood intravenously from N.C.O. Each of these subjects developed clinical attacks of malaria, but neither one exhibited high parasite densities. The parasites were *vivax* type with enlarged erythrocytes and Schüffner's stippling. These infections are still under study.

Both D.E.E. and N.C.O. received chloroquine treatment within 24 hours after diagnosis. D.E.E. had one severe paroxysm a few hours after treatment was initiated; N.C.O. was treated the morning after a paroxysm and had no further fever. Parasites were promptly cleared in each instance, and neither patient has relapsed, to date. Parasitemia levels of both patients were low at the time of treatment (50 parasites or less per cubic millimeter).

Another staff member, H.A., allowed 30 to 50 *Anopheles freeborni* mosquitoes heavily infected with *Plasmodium cynomolgi* subspecies *bastianellii* to feed on him. Eleven and 12 days later he experienced headaches and a general feeling of malaise, and his temperature rose to approximately 100°F. When parasites could not be demonstrated, 10 ml of his blood was injected intravenously into an uninfected monkey. Parasites were demonstrable in this animal 6 days later, proving a subpatent infection in H.A.

A second staff member, C.S.S., allowed himself to be bitten by ten *Anopheles freeborni* heavily infected with *P. cynomolgi bastianellii*. Fourteen days later he experienced a typical malaria paroxysm, and *vivax*-type parasites were easily demonstrable. Treatment with chloroquine was initiated shortly thereafter.

Although circumstances indicated clearly that the malaria in question was *Plasmodium cynomolgi bastianellii*, cross-immunity studies were made. Two monkeys which had spontaneously recovered from infections with this parasite were inoculated with 400 million parasites each from monkey W633, which had received parasitized blood from D.E.E. One of the monkeys showed a transient parasitemia reaching a peak density of 1004 per cubic millimeter indicating nearly complete immunity. The other showed an infection which attained a peak parasite density of 84,800 per cubic millimeter, but the attack was shorter and less severe than the usual *bastianellii* attack. Cross-immunity studies thus supported the belief that the human infections were due to *P. cynomolgi bastianellii*.

The mode of infection of the patients accidentally infected must certainly have been by mosquito bite. Both persons were dealing closely with heavily infected mosquitoes, and it must be acknowledged that the mosquitoes were not being handled with the care and respect accorded mosquitoes infected with known human malarias. The development of the subpatent infection in H.A. and the unmistakable attack, with easily demonstrable parasites, in C.S.S. demonstrated that human malaria can arise from sporozoites of *P. cynomolgi bastianellii*.

Prior to the observations reported here it was known that *Plasmodium knowlesi* is transmissible to man by blood inoculation. This was first established by Knowles and Das Gupta (*3*) and has been confirmed a number of times. *Plasmodium knowlesi* exhibits a quotidian periodicity, and the parasite is morphologically quite dissimilar to the *vivax* type.

The only other successful transmission of a lower-monkey malaria to man known to us was that carried out by Ionescu-Mihaiesti *et al.* (*4*), who transmitted a malaria parasite of the African baboon *Papio babuin* to man and then to *Macaca mulatta*, using infected blood. No full morphological description of the parasite was given, but Ionescu-Mihaiesti *et al.* believed it to resemble *Plasmodium inui*. Sinton *et al.* (*5*) believed this parasite to be more similar to *P. gonderi*, but Rodhain and van der Berghe (*6*) believed it to be a distinct species. The strain was not maintained, due to a shortage of monkeys.

Two unsuccessful efforts have been made to transmit typical *Plasmodium cynomolgi* to man. Sinton *et al.* (*5*) used infected mosquitoes and blood in one patient with general paresis and infected mosquitoes in another. Neither showed any evidence of infection. Coggeshall (*7*), using infected blood and sporozoites, also failed in four attempts to infect patients.

The existence of a *vivax*-type parasite of lower monkeys transmissible to man may have important epidemiological implications. This observation, taken with others, indicates that primate malarias may not have such strict host specificity as is commonly supposed. Particularly significant in the present study is the fact that human infections were induced by sporozoites. Previous human infections with lower-monkey malarias were induced by means of infected blood, so it was not clear whether the human liver would support the pre-erythrocytic stages.

We believe that research on lower-monkey malarias, with particular regard to the possible existence of reservoirs of human infection, or sources of reinfection of man with new strains, is necessary as an essential adjunct to the present malaria eradication program.

Note added in proof: After this report was submitted for publication we learned from Leon Schmidt, director of the Christ Hospital Institute for Medical Research, Cincinnati, Ohio, that one of his staff members (R.G.) had experienced an attack of malaria shortly after the second accidental infection was diagnosed at the Memphis laboratory. Parasitized blood from the Christ Hospital patient produced a typical *cynomolgi*-type infection in a rhesus monkey. Typical *Plasmodium cynomolgi* and the subspecies *bastianellii* are present in the Cincinnati laboratory.

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