ARREST REACTION WITH CONCOMITANT SPIKE AND WAVE AFTERDISCHARGE FOLLOWING THALAMIC STIMULATION IN CONSCIOUS JUVENILE MONKEYS WITH Al $(OH)_3$ FOCAL PREMOTOR LESIONS*

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(Received on June 30, 1981)

Summary : The behavioural and electroencephalographic responses resulting from stimulation of a specific area in the nucleus medialis dorsalis (nMD) of unanaesthetized juvenile Rhesus monkeys is described. A behavioural arrest reaction, lack of awareness and motor inhibition with concomitant $2\frac{1}{2}$ to 3Hz spike and wave EEG afterdischarge patterns were obtained by stimulation of the nMD in monkeys with bilaterally symmetrical aluminium hydroxide Al $(OH)_3$ lesions in anterior premotor cortical areas.

Key words: absence spike and wave discharge thalamic nuclei Rhesus monkey

INTRODUCTION

Hunter and Jasper (1) showed that stimulation of the intralaminar thalamic nuclei in unanaesthetized cats resulted in fixation of the eyes and arrest of movement, resembling the behavioural components of absence seizures (petit mal epilepsy). During the seizures, a bilaterally synchronous 3/sec spike and wave pattern was simultaneously obtained in the EEG and was associated with a similar type of wave form in the thalamus. In previous studies on pentobarbital anaesthetized rhesus monkeys, we obtained typical 3/sec spike and wave responses in response to stimulation of a specific area in the nucleus medialis dorsalis (nMD) (2). This report describes the behavioural response and associated EEG pattern following stimulation of the nMD in unanaesthetized juvenile (pre-adolescent) monkeys.

MATERIAL AND METHODS

20 male colony reared Rhesus monkeys (2.5 - 2.8 *kg*), 14 to 18 months old, were anaesthetized with sodium pentobarbital, 35 *mg/kg* i.p., after overnight fasting. The

*Contribution No. 623 from CIBA-GEIGY Research Centre, Bombay

head and an area in the vicinity of the saphenous vein were shaven and the skin disinfected with mercurochrome solution, 2% w/v. All operative procedures were carried out under aseptic conditions with the animals positioned in a stereotaxic instrument. Additional amounts of pentobarbital generally 5 *mg/kg* i.v., were injected as required. The cranial surface was exposed and small burrholes were made bilaterally, at requisite sites, with No. 5 (Maillefer) carbide dental burrs. The dura was punctured with a sterilized surgical needle and monopolar stainless steel electrodes, 0.25 *mm* in diameter insulated except at the tip, were carefully implanted in the left and right hemispheres at the coordinates A 8; L 0.5 and 1 and H+8 to+9 (Olszewski, 1952). This region in the nucleus medialis dorsalis was previously established, as the optimal location for obtaining electrically induced spike and wave EEG patterns in acute experiments on pentobarbital anaesthetized juvenile monkeys (2). Olszewski has shown that changes in dimensions of different parts of the brain and skull, within certain limits, do not affect the position of the thalamus.

A sterile, aqueous solution of aluminium hydroxide gel (Fischer A 583), was used for intracortical injections, one *ml* containing 48 *mg* of aluminium and 50 *µg* of alcian blue. 50 *µl* of the suspension was injected into each hemisphere at symmetrical anterior premotor cortical sites at coordinates of A 30; L 3 to 6 and H 3 mm below the cortical surface (n=18). No injections were made in another two monkeys. The burrholes were sealed off with a small amount of sterilized gelfoam introduced first, towards the brain surface and then bone wax (Ethicon)* was superimposed to close the craniotomy permanently. Stainless steel screws serving as EEG recording electrodes were inserted into the skull in bilaterally symmetrical positions over the prefrontal motor cortex (A 30, L 6); frontal (A 15, L 6) and parietal areas (A 0, L 7). A reference electrode was placed on the frontal bone over the sinus. Flexible, insulated wires were secured around the base of the screws. The intralaminar thalamic depth electrodes and the wires for EEG recording were soldered to a socket and the entire assembly was permanently fixed to the skull with dental cement. Wound margins were approximated and antibiotic dusting powder applied. Post-operatively, Procaine penicillin, 200,000 units and 1 *ml* of a veterinary injectable solution of liver extract and Vitamin B complex (Livogen, Glaxo)* was given intramuscularly, once a day for 5 days. Monkeys returned to normalcy 2 to 4 hrs following surgery and thereafter were housed individually in spacious cages.

A fortnight after postoperative recovery, testing of behavioural and EEG responses to electrical stimulation of thalamic nuclei was carried out on monkeys adapted to minimal restraint in a primate chair, in a sound attenuated, darkened room, kept at 24°C. These precautions were essential as EEG features were best elicited in relaxed and drowsy animals.

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The leads from the depth electrodes in the thalamic nuclei were connected via a flexible cable to the appropriate outputs of a stimulation isolation unit of a Grass Model S88 stimulator. Square wave pulses of .1 or.2 msec duration, 5 to 10V and a frequency of 30 Hz, given for 10 secs, was used for thalamic stimulation. Low frequency stimulation from 3 to 15 Hz did not yield any responses. Higher voltages were not used as some monkeys showed clonic jerks. An 8 channel Grass Model 78D Polygraph was used for recording the EEG. Stimulations were done hourly between 8.00 a.m. to 4.30 p.m. and behavioural patterns and characteristics and duration of the post-stimulatory spike and wave EEG patterns were established. The response testing sessions were carried out fortnightly, until the characteristic behavioural "arrest" reaction and concomitant spike and wave EEG patterns developed into a reproducible pattern of events.

Other physiological variables such as the electromyogram (EEG) electrooculogram (EOG), electrocardiogram (ECG) and respiration were recorded in 10 out of 18 monkeys during testing sessions according to methods described previously (3).

Histological confirmation of the thalamic sites of stimulation was done when the monkeys were about 2½ years old and could no longer be employed for drug testing, as thalamic stimulation resulted in clonic seizures. Brains were removed and fixed with 10% formalin. Electrode tip placement was detected by 50 μm sections.

RESULTS

Stimulation of the nMD of the thalamus resulted in three distinct and reproducible behavioural events. These characteristic behavioural changes were seen only in those monkeys (n=18) with $Al(OH)_3$ implants in premotor cortical areas and consisted of :

Arrest reaction : During a state of relaxed wakefulness, nMD stimulation with 10 sec trains of 30 Hz, 0.1 msec pulse duration and voltage varying from 5V to 10V caused a sudden interruption of all ongoing activity and immobility. Minor adversive movements of the head and eyes towards the side of stimulation were seen. The normal alert appearance was replaced by a facial expression which can best be described as "blank" or "staring" and unrelated to the environment (Figs. 1 and 2). The post-stimulatory EEG pattern in the awake state consisted of irregular, ill defined spike and wave forms of 2 to 4 Hz which constituted the thalamic afterdischarge pattern.

Lack of awareness : In the drowsy animal, stimulation resulted in widening of the palpebral fissure, immobility, occasional mydriasis, adversive movements of the eyes and head. Rarely, myoclonic movements of the head, extraocular or auricular musculature were observed. Lack of awareness was apparent because the monkeys had no recognition

of approaching visual objects, as judged by the failure to react to a coloured pencil or other familiar object. This behaviour continued into the post stimulatory period and concomitantly, well defined, bilateral synchronous bursts of $2\frac{1}{2}$ to 3 Hz spike and slow wave EEG complexes were seen (Fig. 3). The post stimulatory behavioural and EEG phenomena lasted for a few seconds and on cessation, there was immediate resumption of normal behaviour. The characteristic spike and wave EEG pattern in response to thalamic stimulation, occurred reproducibly during sequential trials after a latent period of 63 ± 5 days following surgery. Such responses could be obtained for a period of about 8 to 10 months and thereafter nMD stimulation resulted in tonic clonic generalized seizures.

Monkeys (n=2) without prefrontal $Al(OH)_3$ lesions showed adversive movement of the head and eyes and myoclonic jerks but remained conscious during nMD stimulation. No spike and wave EEG afterdischarge patterns were observed.

Motor inhibition : It was observed that if the monkeys were engaged in some definitive motor activity prior to nMD stimulation, such as chewing a piece of fruit, all masticatory movements were completely arrested during the period of stimulation. In drowsy animals, lower jaw muscles relaxed and the mouth remained slightly open during nMD stimulation (Fig. 2).

Autonomic changes : Of the autonomic variables recorded, changes, in the order of incidence were seen most frequently in respiration (Fig. 4). The amplitude of respiratory excursions were greatly reduced and at times was almost arrested. Normal respiration (inspiratory and expiratory excursions) occurred after the EEG spike and wave discharge was abruptly terminated (Fig. 5). Changes were almost never observed in the ECG and EOG, whereas the lowered muscle tone during and following nMD stimulation was infrequently registered in the EMG. Mydriasis was occasionally observed.

DISCUSSION

The arrest reaction first described by Hunter and Jasper (1) from stimulation of the nonspecific medial thalamus of unanesthetized cats, is also obtained by nMD stimulation in conscious juvenile Rhesus monkeys. This response occurred only with medium frequency stimulation (30/sec). Motor inhibition also occurred simulataneously.

The results describe a new method for producing behavioural and electrographic features of absence seizures in conscious, juvenile (pre-adolescent) rhesus monkeys, that is currently being utilized in our laboratory. A behavioural arrest reaction together with spike and wave afterdischarge EEG patterns were obtained by thalamic (nMD) stimulation in monkeys with bilaterally symmetrical aluminium hydroxide ($Al(OH)_3$) focal

Fig. 1 : Monkey No. 817 prior to stimulation of nucleus medialis dorsalis (nMD) showing normal alert behaviour.

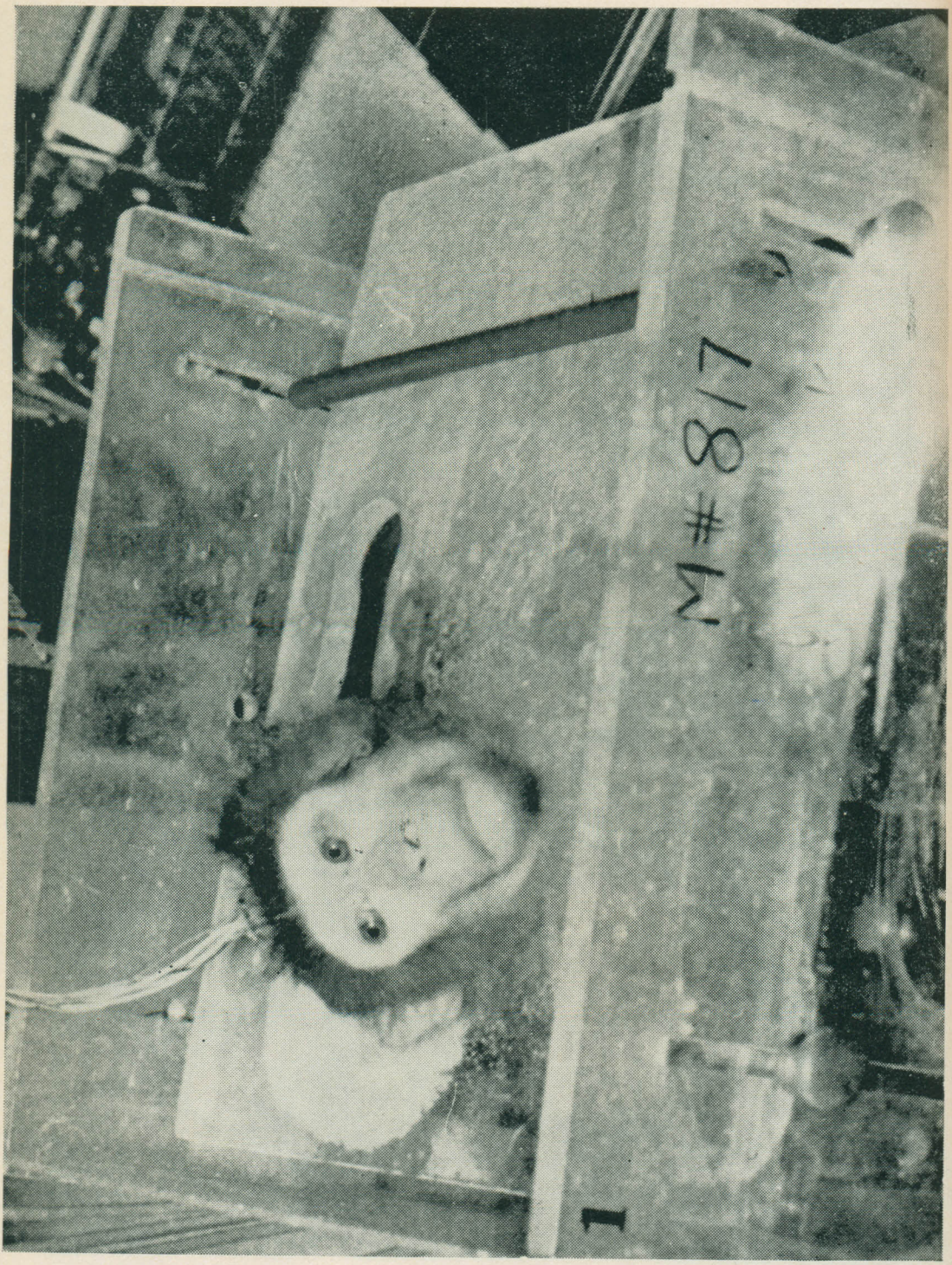

Fig. 2 : Monkey No. 817 during stimulation of pMD. Note that adversive head and eye turning to the right occur together with lower lip

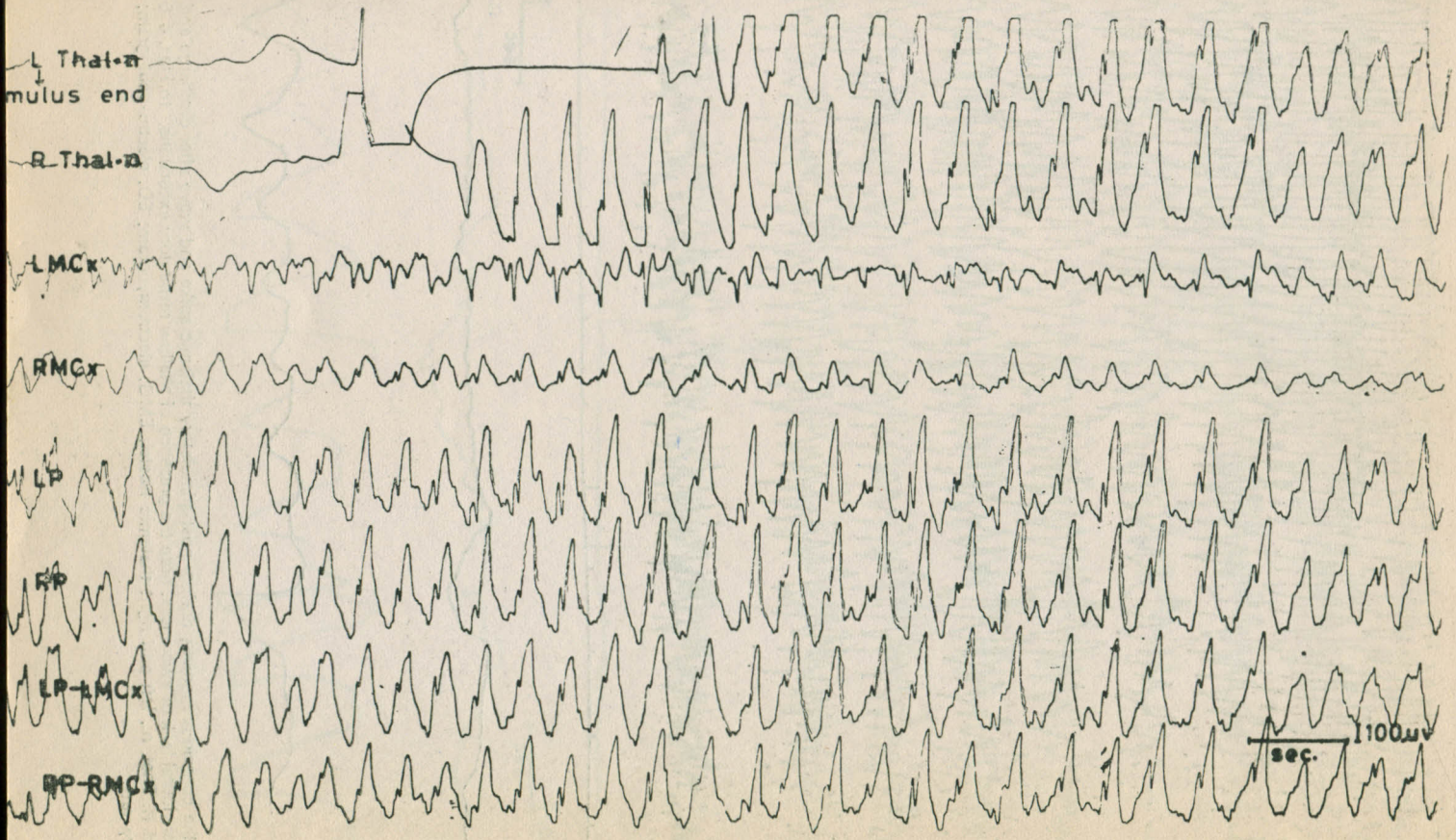

Fig. 3 : Characteristic post-stimulatory thalamic afterdischarge pattern obtained in the EEG of conscious but drowsy monkeys. Stimulus parameters were : 30 Hz, 1 msec, 6V for 10 seconds. The 2½ to 3 Hz spike and wave pattern are best seen in thalamic and parietal leads. Monopolar derivation is used in first six leads. L & RMCx left and right motor cortical areas; LP & RP left and right parietal areas. EEG settings on Grass Model 78D. ½ amp LO FREQ at 1 Hz; ½ amp HI FREQ at 1 KHz; Time constant at 0.3 msec; Pens at 30 Hz; 50 Hz filter, in.

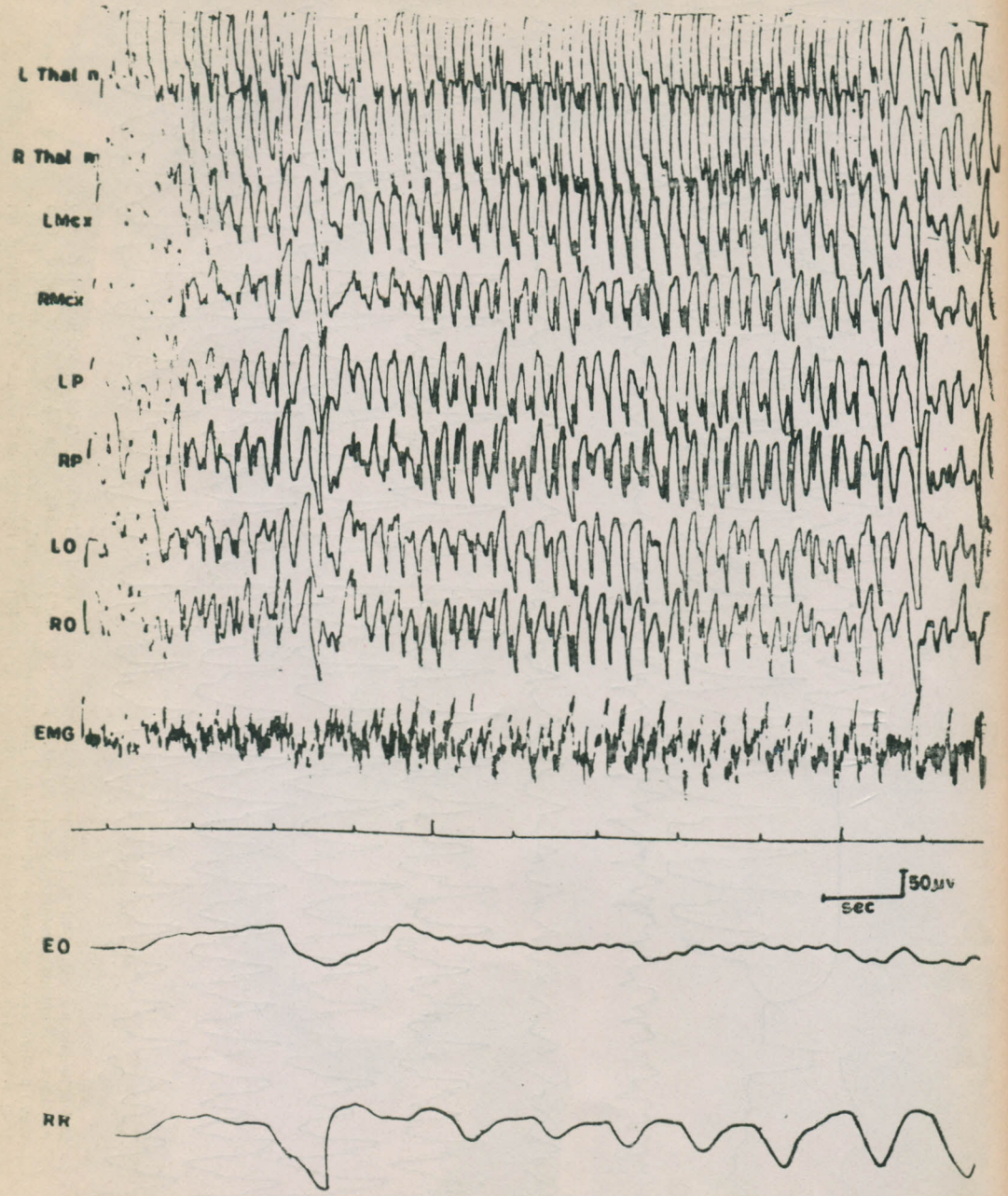

Fig. 4 : In the conscious monkey, during post-stimulatory thalamic spike and wave after discharge and concomitant behavioural lack of awareness, note the presence of shallow respiratory excursions. In Figs. 4 & 5, L. Thal. n and R. Thal. n., left and right thalamic nuclei, EMG, electromyogram; EO, electro-oculogram; RR, respiratory rats.

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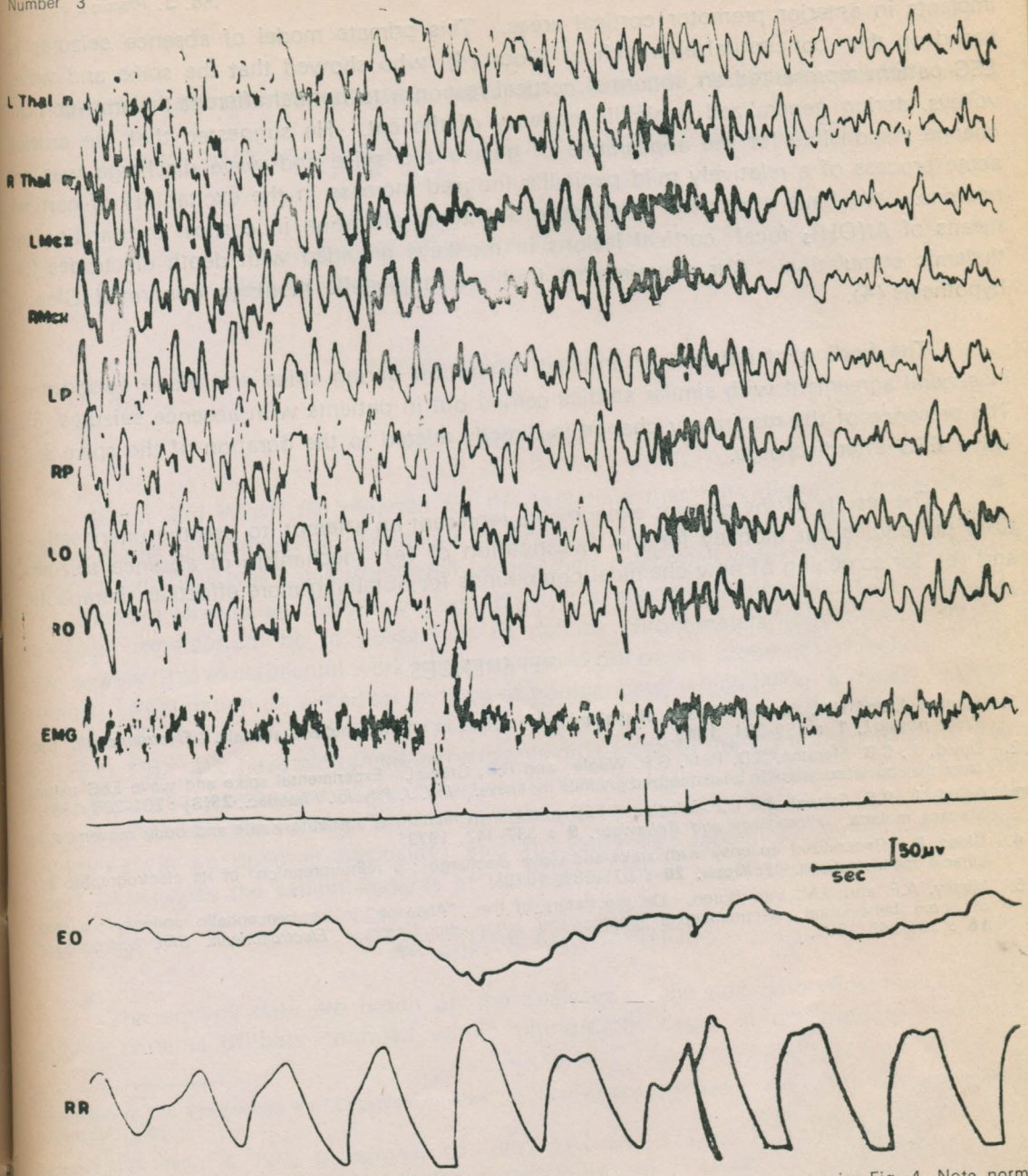

Fig. 5 : Shows continuation of EEG tracing after the spike and wave afterdischarge seen in Fig. 4. Note normal respiratory excursions coincident with disappearance of spike and wave pattern and resumption of normal EEG pattern

implants in anterior premotor cortical areas. This primate model of absence seizures is based on the conceptual approach of Gloor (4), who showed that the spike and wave EEG pattern represented an abnormal cortical response to normal afferent thalamo-cortical volleys, during generalized penicillin epilepsy of the cat. He suggested that the crucial change responsible for the appearance of generalized spike and wave discharge was an acute process of a relatively mild penicillin induced increase in the excitability of cortical neurons. We have attempted to produce a chronic alteration in cortical excitability, by means of $Al(OH)_3$ focal cortical lesions in monkeys provided with depth electrodes for thalamic stimulation. Our experimental findings in juvenile monkeys confirmed Gloor's hypothesis (4).

The finding of reduced or arrested respiration during spike and wave episodes is in general agreement with similar studies carried out in patients with absence seizures (5). The presence of the respiratory change is directly related to the duration of the spike and wave EEG afterdischarge.

The results of the present experiments represent an attempt to use the unanaesthetized pre-adolescent monkey for the investigation of (a) mechanism of absence seizures and (b) for screening of new chemical compounds for obtaining more effective therapeutic agents

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