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STUDIES OF LSD AND CHLORPROMAZINE

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A GOOD deal of clinical research in psychiatry has recently been directed toward the possibility that chemical alterations in nervous structures may be responsible for mental disease or that chemical compounds may aid in restoring normal function in the mentally ill. Both phases of such research have spurred interest in the basic neuropharmacological techniques which may yield relevant information about compounds of current interest.

In our laboratory, compounds of both types have been under investigation.[4, 7] Of those which may give information as to chemical alterations in mental disease by mimicking the symptoms of such disease, the so-called psychotomimetic compounds, some data will be presented on LSD-25. From investigations of several of the drugs which for lack of more precise terms we call tranquilizing agents or psychotherapeutic compounds, studies of chlorpromazine will be used as an example. From the clinical point of view, it has been hoped that an understanding of the site of action of these compounds might offer suggestive data as to the underlying mechanisms associated with mental disease. Furthermore, finding test systems in which such drugs as LSD-25 and chlorpromazine are active would permit much more rapid evaluation of centrally active drugs in order to select those which may be of value in the clinic.

Certain conditions of importance to neuropharmacological studies must first be considered in undertaking, and interpreting data from, animal experimentation. A major problem in studies of this type is that of control. It is of utmost importance to determine whether the observed physiological or behavioral change induced by the administration of the drug is a specific or generalized phenomenon. For this reason, we have employed, wherever possible, threshold stimuli for eliciting the control response. We have considered it essential to monitor stimuli as to voltage and amperage to eliminate such experimental artifacts as changing characteristics of the electrodes. Furthermore, in an ideal experiment following the changes induced by a drug, responses return to baseline or control levels after the drug has been eliminated. In studies of compounds having a short duration of action, such as pentobarbital or thiopental, it is possible to wait for return of control thresholds. In the case of LSD-25 and chlorpromazine, however, which have rather long durations of action and slow onset of action in the case of LSD-25, this is not practical. To provide controls in such long-lasting experi-

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ments so that drug effect may be distinguished from deterioration of the preparation, we have assessed in the same animal the activity of several pathways simultaneously, or assessed the component parts of the primary test system in comparison to the system as a whole. A further control was the use of agents about which some information as to site of action is available as comparison; for example, pentobarbital.

The dose range to be used represents another important consideration. For each compound, we administered a range of doses to unanesthetized animals of the species in which the definitive work was to be undertaken. Only pharmacological doses were selected for acute experiments, that is doses which produced either the behavioral effect whose mechanisms were under investigation or some measurable physiological concommitant.

From recent studies revealing the marked selective effects of anesthetic agents on various pathways, it has become apparent that studies of drug action must be done on unanesthetized animals. In the experiments to be reported here, several hours were always allowed to elapse after surgery under ether anesthesia so that the ether was completely dissipated before beginning control studies.

All experiments were carried out in unanesthetized, curarized cats under assisted respiration. Body temperature was maintained. Oscillographic and electroencephalographic recordings were taken in the conventional manner. Thresholds were obtained by stimulation of the various sites using a Grass or Tektronic stimulator with appropriate isolation unit. Threshold voltage was obtained for a given response by starting below threshold and administering trains of stimuli or single shocks at gradually increasing voltage until the response was obtained. Before administration of the drug, thresholds for each response were determined a minimum of three times. Preparations with variations in control thresholds or response amplitude were discarded. After the administration of the test drug, periodic evaluations of thresholds were done. These were continued until the stability of the preparation decayed in all systems non-specifically. Electrode placements were verified histologically.

From clinical data and observations as to the general effects of the tranquilizing and hallucinogenic drugs, certain pathways in the CNS appeared most likely to be vulnerable to the agents. The selected systems for study were the EEG arousal and recruiting responses, thalamic conduction in classical afferent pathways, evoked seizures in the rhinencephalon and spontaneous EEG changes.

The known depressant action of chlorpromazine on the central nervous system suggested that the compound might reduce afferent inflow through the central nervous system as a portion of its action on anxiety syndromes in man. The effects of this drug were therefore studied on recovery cycles in

the primary afferent pathway. The classical afferent pathway from sciatic or radial nerve involves: peripheral nerve, synapse in cord or dorsal column nucleus, synapse in the thalamus and projection to the cortex. Although single potentials at capsule and cortex are known to be little affected by anesthetic agents, it has been shown that recovery time in the thalamus is markedly delayed by barbiturates.[8, 11] When paired equal shocks were delivered, the second following the first at intervals from 10 to 400 msec. and the relative amplitude of the second response measured as a percentage of the first, the second response of any pair was much reduced after barbiturate administration. This alteration was noted in the capsular potentials and was indicative of depression in conduction at the thalamic relay rather than below the relay in the lemniscus. In well controlled studies, chlorpromazine and LSD-25 did not alter recovery time in the thalamus, as measured by capsular responses, nor in the cortex (Figure 1). LSD-25 did, however, markedly change the cortical wave form. Pentobarbital administered terminally was able to alter conduction in the thalamus after the administration of either agent.

From the clinical reports of sedation without the depression attending barbiturate administration, many investigators have theorized that chlorpromazine must have its therapeutic effect by suppressing the reticular activating system. Moruzzi and Magoun [12] first elaborated the role of the reticular formation in the sleep-wakefulness continuum by stimulation of this area in the absence of central anesthesia to produce EEG recordings similar to wakeful patterns. Lindsley *et al.*[9, 10] demonstrated that lesions in this system produce somnolence while stimulation in the animal with chronically implanted electrodes produced behavioral and electroencephalographic arousal. French *et al.*[2] and Arduini and Arduini [1] also demonstrated that the relay from the lemniscal pathways into the reticular formation with conduction upward was affected by low doses of barbiturates, doses which could be considered sedative rather than anesthetic. To test the hypothesis that this system is also sensitive to chlorpromazine, a preparation was employed measuring the functional reactivity of the reticular activating system and of thalamo-cortical circuits.

Three pathways were studied simultaneously. The primary response, the EEG arousal reaction, was elicited by stimulation at 150 per second of the brain stem reticular formation, diffuse thalamic projection system, and the sciatic nerve in successive control series. Recordings were taken in thalamus, caudate nucleus, or other deep areas and at various sites on the cortex. To indicate cortical reactivity, recruiting responses were evoked at the same recording sites by low frequency stimulation (seven per second) of the diffuse thalamic projection system. Typical responses at threshold voltages are illustrated in Figure 2. Criteria for EEG arousal included promptness of onset

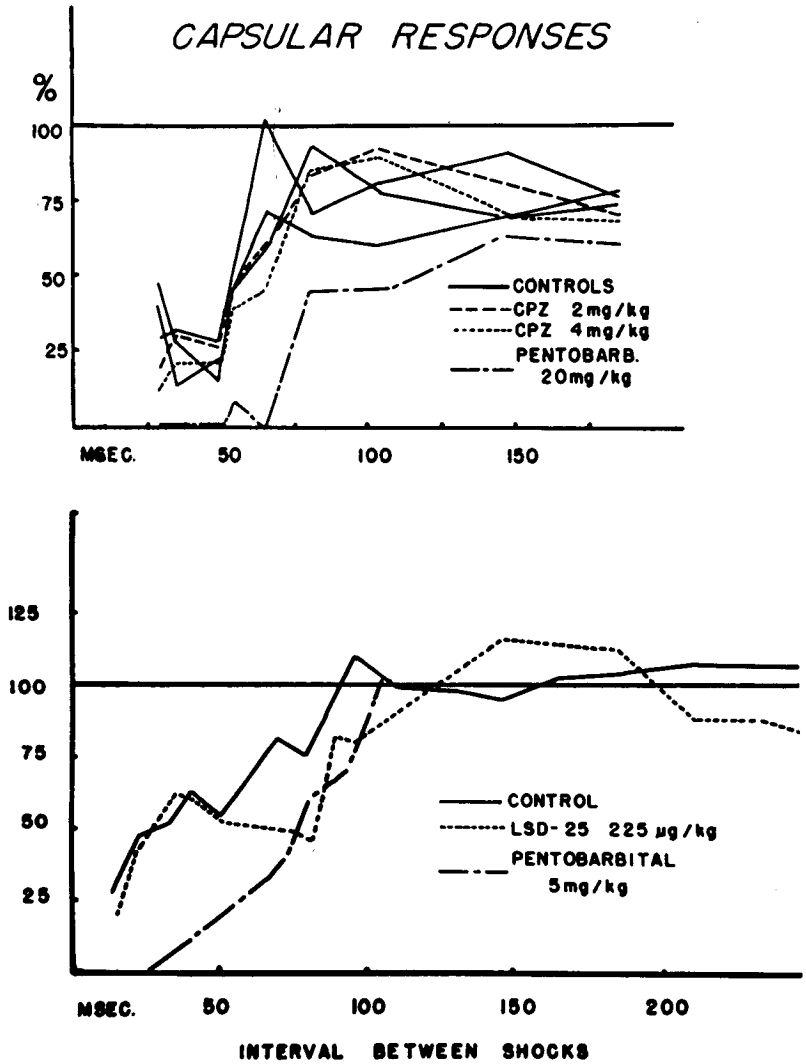

FIGURE 1. Capsular responses following stimulation with paired shocks to the central end of the contralateral radial or sciatic nerves. Abscissa indicates interval between members of any pair of shocks. Ordinate indicates percentage response of second shock of any pair with respect to the first of the same pair. A. Typical record before and after administration of chlorpromazine to the unanesthetized curarized cat. B. Typical record before and following administration of LSD-25 to the unanesthetized curarized cat.

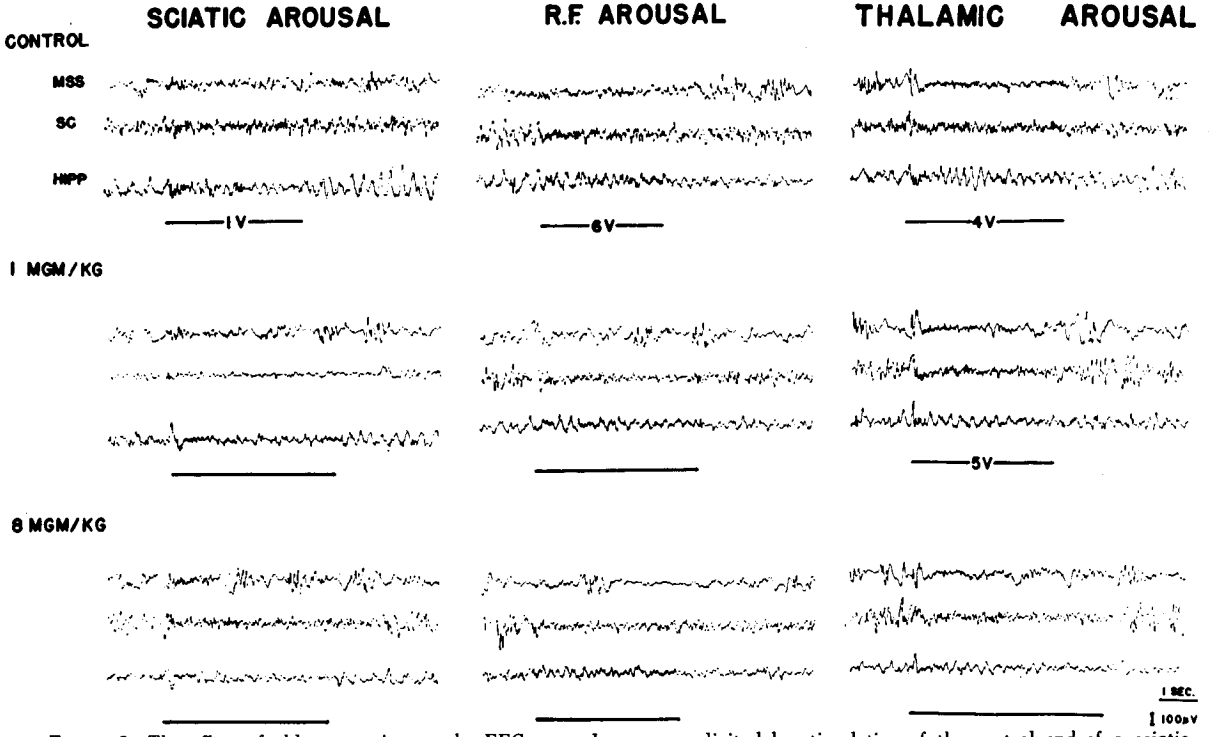

FIGURE 2. The effect of chlorpromazine on the EEG arousal response elicited by stimulation of the central end of a sciatic nerve, of the brain stem reticular formation, and of the diffuse thalamic projection system at 150 per second. MSS—medial suprasylvian gyrus, SC—sensory cortex, Hipp—hippocampus. Solid bars indicate voltage and duration of stimulation.

of the characteristic response on stimulation and persistance of the response beyond the termination of the stimulation. The responses noted in isocortical leads, the top two in the figure, were the conversion of high voltage slow activity to low voltage fast activity. The rhinencephalic arousal response, as previously described by Green and Arduini [3] is depicted in the third tracing, and was characterized by the induction of high voltage synchronous activity. The effect of chlorpromazine at one to eight mgm. per kgm. was a slight but definite elevation in the threshold of isocortical arousal. The rhinencephalic arousal response was more affected. These changes were independent of the source of EEG arousal.

The simultaneous measurement of the recruiting response following stimulation of the diffuse thalamic projection system is illustrated in Figure 3. The recruiting response in cortex (top line) and diencephalic leads consists of characteristic waves which increase in voltage with successive similar shocks and then in response to later stimuli wax and wane in amplitude. Chlorpromazine, at one mgm. per kgm. slightly increased this response, indi-

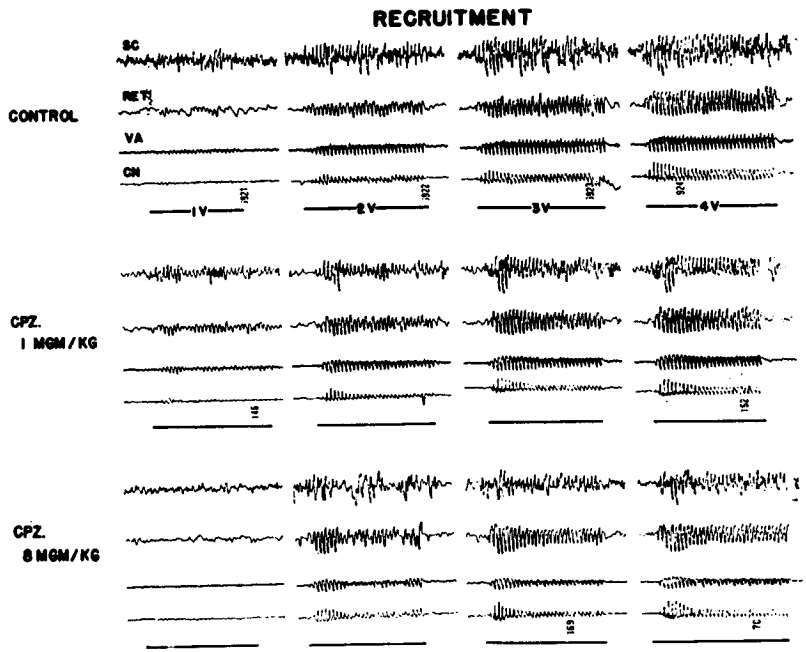

FIGURE 3. The effect of chlorpromazine on the EEG recruitment response elicited by stimulation of one of the nuclei of the diffuse thalamic projection system at 7.5 per second. SC—sensory cortex, RET—nucleus reticularis of the thalamus, VA—nucleus ventralis anterior of the thalamus, and CN—caudate nucleus. Solid bars indicate voltage and duration of stimulation.

cating that the slight depression of the arousal reaction shown in the tracings of Figure 2 was not due to depression of cortical or subcortical responsiveness to the inflow from the reticular formation. At eight mgm. per kgm. the threshold of the recruiting response was depressed to two volts, but this was only a small change and responses at submaximal voltages remained high in amplitude.

LSD-25 elevated the threshold by one-half to one volt for the EEG arousal response elicited by sciatic, reticular formation or thalamic stimulation. Typical effects of LSD-25 are shown in Figure 4. It is to be noted that this shift was comparable to that seen with effective doses of chlorpromazine. An important difference was that the rhinencephalic response was not altered after LSD-25 to the degree seen after chlorpromazine.

The threshold for eliciting the recruiting response was unaltered by 50

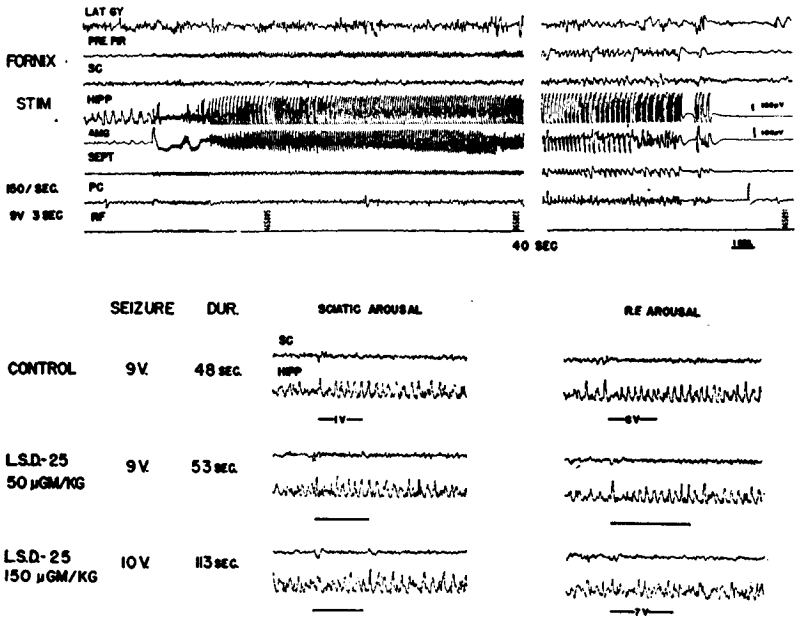

FIGURE 4. Effect of LSD-25 on rhinencephalic seizures evoked by stimulation of the fornix at 150/sec. and on the EEG arousal response elicited by stimulation at 150/sec. of the central end of a sciatic nerve and of the brain stem reticular formation. LAT GY—Lateral gyrus, PRE PIR— prepiriform cortex, SC—sensory cortex, HIPP—hippocampus, AMG— amygdala, SEPT—septum, P C—reticular formation at the level of the posterior commissural outflow, RF—reticular formation at the level of the red nucleus. Lower left hand corner: compilation of thresholds and durations of seizures evoked. Control = average of 4, after 50 micrograms of LSD-25 = average of 2 and after 150 micrograms of LSD-25 = average of 2.

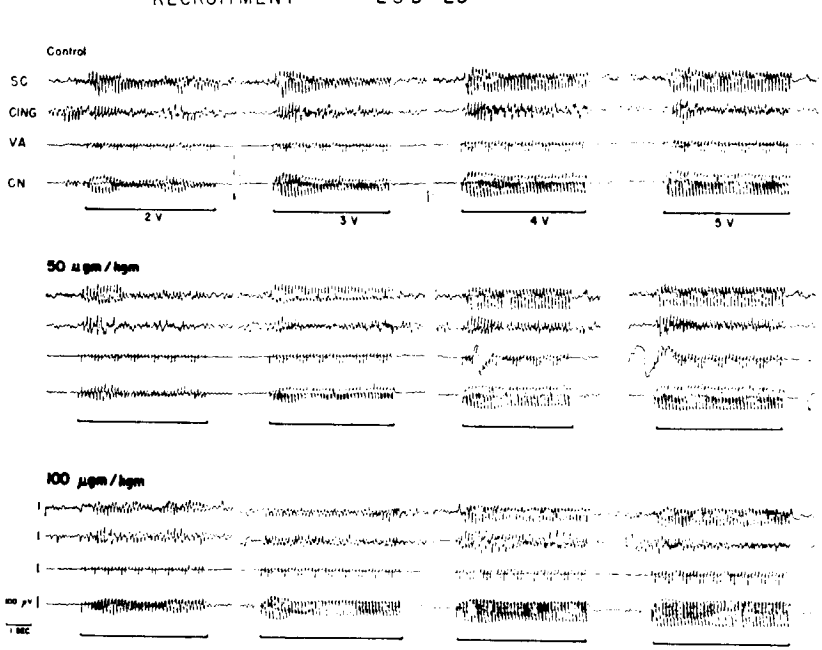

FIGURE 5. Effect of LSD-25 on the EEG recruitment response elicited by stimulation of nuclei of the diffuse thalamic projection system at 7.5 per second. SC—sensory cortex, CING—cingulate gyrus, VA—nucleus ventralis anterior of the thalamus and CN—caudate nucleus. Solid bars indicate voltage and duration of stimulation.

and 100 micrograms per kgm. of LSD-25 (Figure 5). However, the amplitudes of the cortical responses at threshold were depressed.

In contrast, Figure 6 illustrates the sensitivity of this type of preparation to pentobarbital. The recruiting response depicted on the left was enhanced by five mgm. per kgm. of pentobarbital. On the other hand, the threshold for the EEG arousal response elicited by stimulation of the reticular formation was elevated from three volts to more than 10 volts after five mgm. per kgm. of the drug. It is to be emphasized that this dose of pentobarbital is one-sixth to one-seventh the anesthetic dose, with little effect on the unanesthetized animal. The doses of chlorpromazine and LSD-25, in contrast, cause discernible changes in overt behavior in unanesthetized animals.

From the experimental finding that EEG arousal in the rhinencephalic areas was altered by chlorpromazine and from clinical reports describing LSD-25 intoxication, it was thought that the rhinencephalic system should be investigated for possible sensitivity to these agents. Seizures were produced in the limbic system by stimulation of the precommissural fornix.

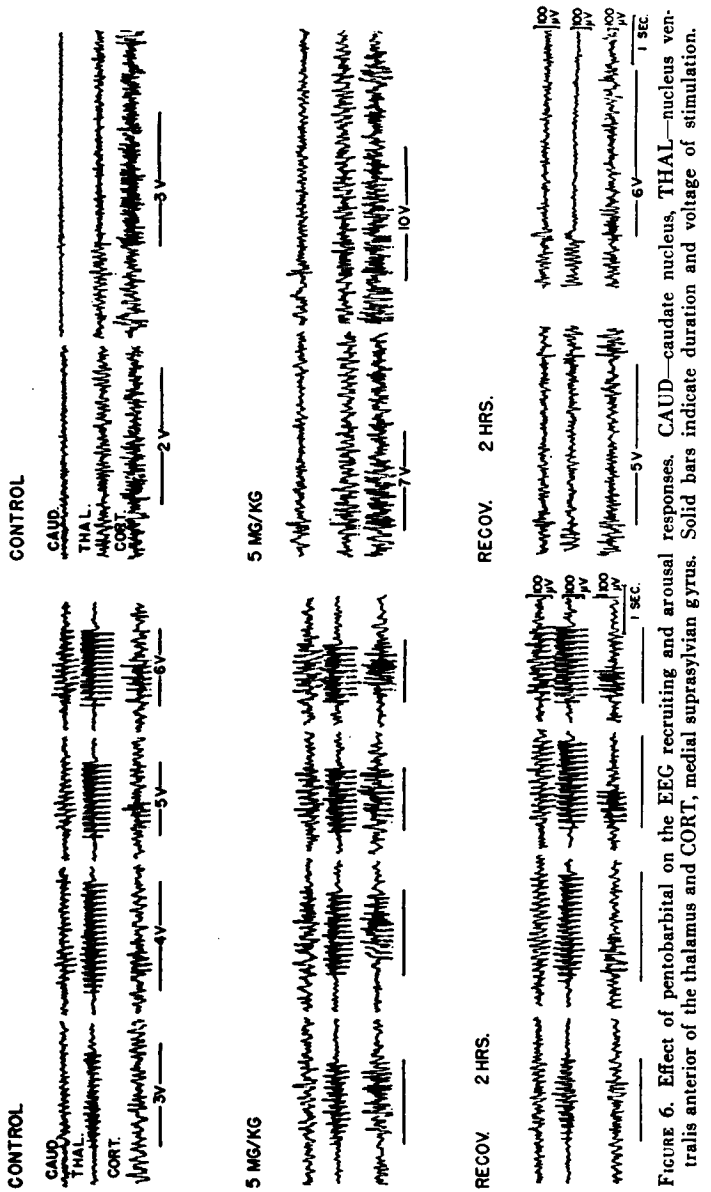

FIGURE 6. Effect of pentobarbital on the EEG recruiting and arousal responses. CAUD—caudate nucleus, THAL—nucleus ventralis anterior of the thalamus and CORT, medial suprasylvian gyrus. Solid bars indicate duration and voltage of stimulation.

Following threshold stimulation, seizure activity can be recorded from amygdala, hippocampus, septum, cingulate gyrus, and entorhinal areas, but usually not from isocortex. These experiments were designed to measure both threshold and duration of seizures.

A typical seizure response is shown in Figure 4. In the lower left hand corner of the figure is a compilation of the threshold and duration of seizures in a typical experiment. After LSD-25, there was a small change in threshold, from nine to 10 v. after 150 micrograms per kgm., and a gradual, then marked increase in the duration of rhinencephalic seizures.

Chlorpromazine elevated the threshold for seizures in the limbic system, but only at doses which markedly depressed unanesthetized cats. Pentobarbital again was the most effective agent in altering rhinencephalic seizures (Figure 7). Pentobarbital at 10 mgm. per kgm. raised the threshold at which rhinencephalic seizures could be evoked from three volts to more

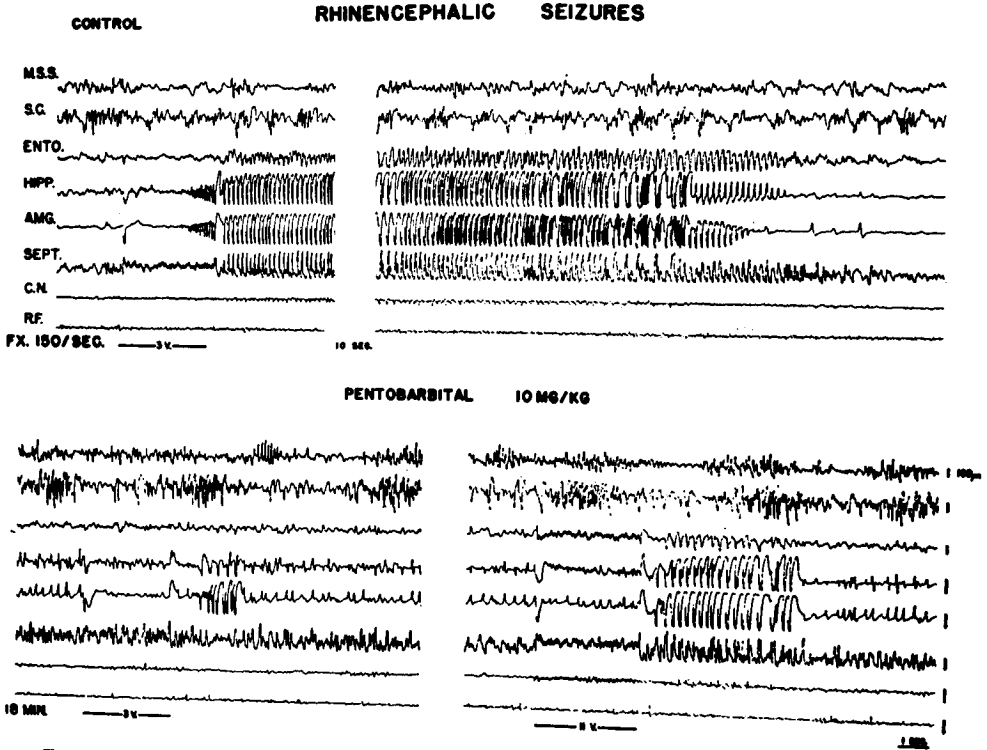

FIGURE 7. Effect of pentobarbital on rhinencephalic seizures evoked by stimulation of the fornix at 150 per second. MSS—Medial suprasylvian gyrus, SC—Sensory cortex, ENTO—Entorhinal cortex, HIPP—Hippocampus AMG—Amygdala, SEPT—Septum, CN—Caudate nucleus, and RF—reticular formation. Solid bars indicate voltage and duration of stimulation.

than 20 volts. After-discharges were seen at 11 volts and at succeeding voltages.

It is to be noted that in the lemniscal and extralemniscal sensory systems and the rhinencephalic responses studied these two potent pharmacological agents, LSD-25 and chlorpromazine, caused only minimal changes. The alterations induced by these agents of opposing activity in the extralemniscal pathways were similar. Rhinencephalic responses were altered in opposite directions. In no pathway was the change commensurate with the striking behavioral changes seen in unanesthetized animals at the same doses of the two agents. In contrast, pentobarbital showed striking activity in all the above systems. A more detailed discussion of the relationship of these data to effects on the nervous system reported by other workers will be published elsewhere.[5, 6]

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