ELECTROGRAPHIC ALTERATIONS INDUCED IN LIMBIC AND SENSORY SYSTEMS DURING INDUCTION OF ANAESTHESIA WITH HALOTHANE, METHOXYFLURANE, DIETHYL ETHER, AND ENFLURANE (ETHRANE)*

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SUMMARY
In the present study of the effects of four volatile agents, diethyl ether, methoxyflurane, halothane and enfurane on the brain, a definite spontaneous activity pattern of various subcortical structures indicating onset of analgesia could be established only for enfurane. It consisted of high voltage spiking activity separated by periods of burst suppression of various durations. Brief periods of high voltage spiking occurred with the other three agents, during induction, regardless of chemical structure. An increased susceptibility of the structures of the limbic system to the effect of halogenated compounds was demonstrated.

The effects of anaesthetic agents on electrical brain potentials were observed by Fleischl von Marxow (1890) shortly after the discovery of their existence in experimental animals. Several decades later when more powerful amplifiers became available, Berger was able to record the cortical activity through the intact skull in humans. He also reported changes which resulted from administration of sedatives to his patients (Berger 1931). Gibbs, Gibbs and Lennox (1937) confirmed these findings and suggested electroencephalography as a tool for monitoring the depth of anaesthesia during operations. This was not possible until in the late forties adequate shielding permitted the use of electronic equipment in the operating rooms. Faulconer, Bickford and their associates (1960, for review) systematically investigated the e.g. of patients anaesthetized for surgery and delineated definite patterns from the preoperative state of wakefulness through all levels of surgical anaesthesia as defined by clinical signs. They did not suggest that the observed modifications of cortical activity by anaesthetic agents offered an adequate explanation for the mode of action of general anaesthetics.

The participation of subcortical structures in the mechanism underlying the anaesthetic state first became apparent when Moruzzi and Magoun (1949) discovered the importance of the reticular formation in the maintenance of wakefulness and sleep and later its ability to respond to stimulation of all sensory modalities (Magoun, 1950). When it was demonstrated that these responses were abolished by barbiturates or ether, the neural basis of the anaesthetic state was proposed (French, et al., 1953). The fact that responses of the mid-line thalamic nuclei, which also receive input from all sensory modalities, are modified by general anaesthetics (Denavit, 1963) cannot be overlooked. The role of certain entorhinal areas in arousal and integration of functions with the reticular formation was stressed by Adey, Dunlop and Sunderland (1958). Brazier suggested (1963) that the limbic system be included in further investigations.

To the best of our knowledge there is no information about the continuous changes in electrical activity of subcortical structures from wakefulness till onset of anaesthesia comparable to those observed in the cortex by Faulconer and Bickford (1960). Therefore, a systematic study of the effects of agents used in the past and present practice of anaesthesia on spontaneous activity in structures representative of the three principal systems is appropriate at this time.

Experiments were conducted on chronic preparations (i.e., animals with permanently implanted electrodes) in which onset of analgesia can be determined without difficulty. This is not possible in paralysed acute preparations, frequently used in neurophysiological studies. Furthermore, a greater number of structures can be monitored

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simultaneously and anaesthesia can be administered in a manner closely resembling that used in the operating theatre. In the experiments reported below, volatile compounds were investigated and findings compared.

METHODS
Eight adult cats (average weight 4.5 kg) were utilized. Under barbiturate anaesthesia stainless steel screws were placed in the skull over the frontal sinus for grounding and over frontal (sensorimotor), parietal, and occipital cortices. Bipolar concentric stainless steel electrodes were stereotaxically introduced into subcortical structures according to the coordinates of Snider and Niemer (1961). The electrodes were connected to a miniature socket which was then secured to the skull with acrylic resin. These depth electrodes were placed in nucleus ventralis postero-lateralis of the thalamus (somatic primary relay nucleus), midbrain reticular formation, n. centrum medianum, n. dorsalis medialis, n. amygdalae, and formatio hippocampalis ventralis.

Agents investigated were diethyl ether and three more recently developed halogenated compounds: methoxyflurane, halothane and enfiurane (Ethrane). Each agent was vaporized in a Copper Kettle attached to an anaesthesia apparatus provided with an infant circle absorber. A high flow of air and oxygen 2:1 l./min was delivered in a semiclosed system via a mask specially designed for cats.

Experiments were conducted at weekly intervals with the sequence of agents at random. Three to four weeks were permitted to elapse from the date of implantation to the first trial. The animals were not premedicated and were breathing spontaneously. End-tidal carbon dioxide concentration was monitored with a Beckman Infrared Gas Analyser and the electrographic activity of the brain was recorded continuously on a Grass Model 78 polygraph.

At the conclusion of the series, the animals were sacrificed and electrode placements verified histologically.

RESULTS
Preliminary trials were conducted with each agent on two or three animals to determine the lowest concentration capable of producing either behavioural or electroencephalographic changes. In the subsequent experiments, induction was started with a somewhat lower concentration and gradually increased until the animal did not respond to a painful stimulus (pinch to a hind paw).

Halothane.
Concentrations lower than 5–6% had little effect on e.e.g. patterns and the animals resisted the face

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**Fig. 1.** Halothane

(A) Control tracing before administration of halothane.
(B) 6% halothane administered for 1½ min; note “spindles” in n. amygdalae (3rd line) and increased amplitudes in all tracings but the reticular formation (5th line). Cat still responding to pinching of hindpaw.
(C) Tracing obtained at 8 min from start, 8% halothane given last 2 min; onset of analgesia.

(Cx=cortex, VPL=n. ventralis postero-lateralis of the thalamus, AMYG=n. amygdalae, HIPP=hippocampalis dorsalis (formatio), RET. F.=reticular form, CM=n. centrum medianum, DM=n. dorsalis medialis of the thalamus; abbreviations same for all figures.)
mask. First changes observed within one minute of administration of 6% halothane were in the n. amygdalae: a rhythmic 16-20 Hz sinusoid type pattern of alternately increasing and decreasing amplitude, resembling barbiturate "sleep spindles". Twenty to thirty sec later, slowing and increased amplitude appeared in the n. ventralis postero-lateralis (VPL) and in the cortex (fig. 1B). Slight slowing and increase in voltage was observed in n. centrum medianum (CM) and n. dorsalis medialis (DM). Increase in amplitude and occasional spikes were observed in the hippocampus. The reticular formation was relatively unaffected. The animals still responded to pinch of the hind paw. With an increase in halothane concentration to 7% and 8% over a period of 6-8 min the animals no longer responded to a painful stimulus and the e.e.g. record was characterized by an overall loss of fast activity in all leads (fig. 1C). The sinusoid pattern previously observed in n. amygdalae persisted but was diminished in amplitude. The slow waves in the cortex and thalamic structures were also of lesser amplitude. While the spike activity of the hippocampus continued, low voltage high frequency patterns disappeared.

**Enflurane.**

The first changes in e.e.g. activity following enflurane were observed within approximately 5 min of induction with a 4% concentration (fig. 2B). There was loss of fast activity in all structures including reticular formation; high voltage activity and spiking was also apparent, with occasional burst suppression. The animals were quiet, but still responsive to painful stimulation. During the next 15 min with the concentration raised to 5%, high voltage slow activity dominated the record followed later by a marked increase in rhythmic slow waves which then progressed to a spike activity. Further increases in enflurane concentrations to 6% resulted in appearance of bursts of high amplitude spikes separated by short periods of isoelectric silence (fig. 2C). At this point the animals finally became unresponsive to the stimulus.

**Ether.**

Inhalation of ether, starting with 2.5% was tolerated by all animals without marked struggling or respiratory irritation; it was possible to proceed with the induction rapidly, so that within approximately 10 min all animals were receiving a 10% concentration. At this time a pattern of higher amplitude was observed in the cortex and VPL, while some slowing with decreased voltage occurred in the structures of the limbic system (fig. 3B). A few minutes later, although the animals were still responsive to pain stimulus, some synchronization was observed in cortex and VPL. While the concentration of ether had to be increased to 20-25% in order to achieve analgesia, the above described patterns did not persist: the high amplitude, slow activity was replaced...
by faster frequency and subsequently lower voltage, until patterns very similar to control tracings were recorded (fig. 3c).

**Methoxyflurane.**

Induction with methoxyflurane was very slow. Starting with a 0.5% concentration, and increasing gradually to 3.5%, analgesia was seldom obtained in less than 35–40 min. First changes, observed after about 15 min with 1.5% methoxyflurane, were increase in amplitude in the limbic system, particularly in the hippocampus and later occasional synchronization in the cortex (fig. 4b). Slowing in all structures, with simultaneous loss of superimposed fast activity was usually recorded when a 3% concentration was administered for several (about 10) min, i.e., 30–40 min from the beginning of the experiment. Later, occasional spikes could be seen in the cortex and VPL and considerably more frequently in the limbic structures. While these patterns

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**Fig. 3. Ether.**

(A) Control tracing.

(B) 10 min from beginning of inhalation of ether in gradually increasing concentrations to 10%. Most noticeable changes in cortex and VPL and to a lesser extent in limbic structures. No analgesia as yet.

(C) 20 min from start; concentration now increased to 20% and inhaled by the animal for about 5 min. Onset of analgesia; note similarity of tracings in all leads with control tracing.

**Fig. 4. Methoxyflurane.**

(A) Control tracing.

(B) 15 min of administration of methoxyflurane, last 1 min 1.5%; no analgesia.

(C) Further increase of concentration of methoxyflurane; this record at 40 min, last 20 min 3%. No response to a painful stimulus.
were gradually developing, the animals were at first still responding to painful stimulation, later the response became sluggish and finally disappeared entirely.

DISCUSSION
Inhalation anaesthetics are distributed throughout the body and act diffusely upon the organism. Certain organs appear to be more sensitive to their action than others. It is believed that in the central nervous system the reticular formation and the nuclei of the diffuse thalamic projection system are particularly susceptible to general anaesthetics (Arduini and Arduini, 1954; French, Verzeano and Magoun, 1953). In our experiments, spontaneous activity of these structures was the least affected of all areas monitored. A definite pattern indicating onset of analgesia was observed only with enflurane. It consisted of groups of high voltage spikes separated by brief isoelectric periods, usually preceded by hypersynchrony. This seizure activity was spontaneous and was not directly related to any type of sensory stimulation. We have not observed involuntary movements of extremities nor muscle twiching during induction with this agent.

The changes observed with ether were only minimal in spite of the high concentrations administered for a prolonged period of time. It would therefore seem that in cats a state of analgesia does not precede loss of consciousness as reported for humans by Artusio (1954).

E.g. pattern changes produced by the halogenated compounds demonstrate a greater sensitivity of the structures of the limbic system to these agents. The first changes produced by methoxyflurane were observed in the hippocampus and n. amygdalae long before analgesia was established (fig. 4b). Later, when analgesia was achieved, spiking occurred both in the structures of the sensory and limbic systems, but to a much lesser extent in the former than in the latter. These findings were consistent in all animals.

The rapid onset of a sinusoid pattern in n. amygdalae observed during all experiments after only a brief period of inhalation of halothane was striking. Similar patterns can be elicited in non-anaesthetized cats by olfactory stimulation or stress (Gloor, 1960). Presumably, all volatile anaesthetics are olfactory stimulants so it is not clear why only halothane should have produced this effect in the present study. All animals were handled gently during all experiments and administration of halothane was not any more stressful than inhalation of the other agents.

With enflurane the first changes were in most instances observed almost simultaneously in all structures, however, with a slight precedence in the limbic system, discernible only upon close scrutiny. The onset of analgesia was marked by high voltage spikes separated by isoelectric periods, originating in the hippocampus and spreading rapidly to the other structures.

It is known that hippocampus and some other rhinencephalic structures have an exceedingly low threshold to seizure discharges (Green, 1960). It is also known from the first reports by Krantz (1969, for review) that fluorine substitution in certain hydrocarbons resulted in compounds with stimulant rather than depressant properties. Convulsive seizures and spike and dome patterns were not infrequent observations and eventually one of his compounds was introduced in psychiatry as a possible substitute for electroshock treatment (Krantz et al., 1957).

In a recent comprehensive study of all inhalation agents, Joas, Stevens and Eger (1971) correlated the chemical structure (ether v non-ether) with abnormal cortical activity of dogs. Based on their own observations and on reports from the literature, they concluded that e.g. seizures are related to the ether linkage in the molecule. In the present study, spiking occurred during inhalation of all four agents investigated. With diethyl ether and halothane, a non-ether, spikes appeared briefly, early during induction, before onset of analgesia. With methoxyflurane, a halogenated ether, a similar pattern was observed at about the time analgesia was achieved. The only agent which produced seizure activity was enflurane, as discussed above.

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REFERENCES


