Differential Effects of Anesthetics on Mesencephalic Reticular Neurons:

I. Spontaneous Firing Patterns

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To obtain further information about the neuronal mechanisms involved in the state of anesthesia, long-term microelectrode recording of activity in the mesencephalic reticular formation in the cat was undertaken. Results were subjected to computer analysis. In most units in the mesencephalic reticular formation (32 of 37 studied) spontaneous firing rates decreased as anesthesia deepened. However, the susceptibility of the spontaneous activity of the units to depression by anesthetic agents varied widely from unit to unit. Two types of changes in spontaneous firing patterns were produced by anesthetics: the "grouping" type and the "tonic" type. The "grouping" pattern was seen more often during sodium thiopental and halothane anesthesia, whereas the "tonic" type was more characteristic of nitrous oxide and diethyl ether anesthesia. In two of 37 units, synchronization between mesencephalic reticular formation unit firings and sharp positive waves of the corticogram was observed during the deep stage of sodium thiopental anesthesia. (Key words: Mesencephalic reticular formation units; Nitrous oxide; Diethyl ether; Halothane; Sodium thiopental.)

SINCE Moruzzi and Magoun[1] first recognized that the process in EEG and behavioral arousal depended upon excitation of the central brain stem, the characteristic convergence and interaction of the different sensory systems within it have been investigated by several authors.

Both French *et al.*[2] and Arduini and Arduini[3] showed that the multisynaptic reticular formation was more susceptible than the direct afferent pathway to anesthetic depression.

In contrast to this evidence, the long-latency responses first described by Forbes[4] are known to have enhanced amplitudes during certain stages of barbiturate and ether anesthesia. This finding suggests that the anesthetic state is not a simple process of neuronal depression, but rather reflects various mechanisms involved in terms of inhibition and activation during the course of anesthesia. Moreover, Huttenlocher[5] showed that in most units in the mesencephalic reticular formation (MRF) more spontaneous and less evoked activity occurred during natural sleep with EEG slow waves than during quiet wakefulness. This also suggests that individual neurons in the MRF are not uniformly involved in the processes of wakefulness, sleep, and anesthesia.

The purpose of the present study has been to examine the effects on the MRF at the unit level of different common anesthetics in various doses, by means of long-term microelectrode recording and computer analysis.

Methods

The series included 28 adult cats of either sex, weighing 2.8 to 4.3 kg. The animals were anesthetized in an airtight plastic box which had two ports for administration or elimination of anesthetic gases. Halothane, 3 per cent in oxygen, was administered through one port. After the cat had been anesthetized to the surgical stage, the intratracheal cannula was inserted and respiration controlled via a nonrebreathing system. Before administration of muscle relaxants, the stroke volume of the respirator was adjusted so that the carbon dioxide tension of arterial blood was approximately 40 mm Hg, to eliminate the effect of hyper- or hypoventilation. Air flow was monitored with a pneumotachograph. Arterial blood pressure was also monitored through a catheter inserted into femoral artery. Body temperature was checked by a rectal ther-

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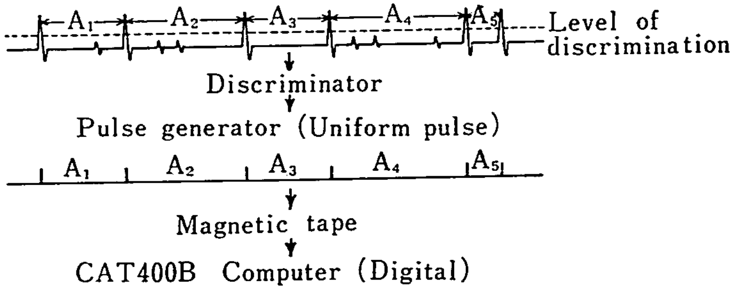
Accepted for publication March 24, 1971. Supported by USPHS grants NS 08961 and 08962.



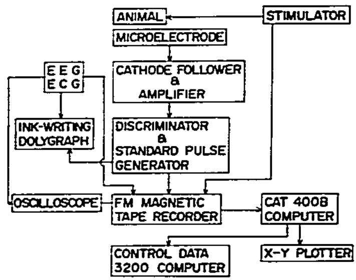


FIG. 1A (*above*) and B (*right*). Schematic diagram of experimental protocol.

mometer. A heating blanket was applied to maintain body temperature constant.

The skull was exposed and a small hole (2 mm in diameter) was made in it. To minimize respiratory and pulsatory brain movements, the dura was not removed. The site of trephination on the skull was AP + 2.0 mm and ML 2 mm in the Horsley-Clark coordinates. Two screw electrodes were fixed to the skull on the areas of the anterior sigmoid gyrus and middle suprasylvian gyri for recording the electroencephalogram.

At the termination of the surgical procedures, procaine, 2 per cent solution, was injected around the wound and the pressure points. Gallamine triethiodide, 20 mg, was injected intraperitoneally, and halothane inhalation was discontinued.

Recording microelectrodes were tungsten or stainless steel wires 250 μ in diameter, polished electrolytically to 0.5–1 μ in tip diameter. AC impedance of electrodes in saline solution varied from 5 to 150 megohms.

Microelectrodes were inserted through the trephinized small hole, intact dura, and several layers of the brain up to the region where the units vigorously responded to changes in room light. This indicated that the electrodes had reached the colliculus superior.[6] After attainment of this response from the units, the electrodes were advanced more slowly. Analysis of each unit's behavior was started only after confirmation of sufficient stability of the action potentials for more than an hour. The activity of every unit studied had an initial negative deflection, which was believed to be the action potential of cell body.[5,6]

Action potentials were led to a cathode follower system and amplified by Grass Model p5 amplifier. Unit activities were monitored with an oscilloscope and, at the same time, led to a discriminator which triggered a standard pulse generator in response to a spike potential. The level of discrimination was adjusted so that spikes of a single neuron could be obtained. The pulse generator output was

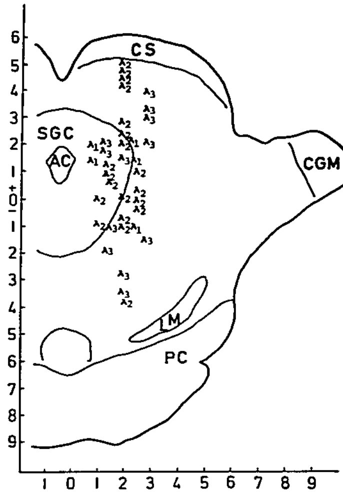


FIG. 2. Approximate positions of the 37 MRF units subjected to analysis. Plots were made at the level of AP +2.0 mm. Units sampled at AP +1.0, +2.0, and +3.0 mm are indicated by A_1, A_2, and A_3, respectively. AC, aqueductus cerebri; CGM, corpus geniculatum mediale; CS, colliculus superior; LM, lemniscus medialis; PC, pedunculus cerebri; SGC, substantia grisea centralis.

monitored on an oscilloscope and on an ink-writing polygraph, together with the electroencephalogram and electrocardiogram. In addition, the unit activity, electroencephalogram, time marker, and stimulus signal were recorded on FM magnetic tape.

The taped records of the behavior of units were analyzed by means of digital computers (CAT 400B and control data 3200). The number of spikes for a fixed time interval (FC), and the interspike interval histogram (IH) were computed, displayed and plotted. Averaged waveforms of the corticogram triggered with unitary spikes were also computed to find a correlation between the EEG and unitary behaviors of the MRF. Schematic diagrams of the experimental arrangements are shown in figure 1.

Regarding depth of anesthesia, the following terminology was applied in reference to clinical anesthesia. Light stage: halothane (H), 0.5 per cent (more than five minutes after inhalation); diethyl ether (E), 3–5 per cent (more than eight minutes after inhalation); nitrous oxide, 75 per cent (more than two minutes after inhalation); sodium thiopental (P), 3 mg per kg (within seven minutes of intravenous injection). Moderate stage: H, 1–1.5 per cent (more than five minutes after inhalation); E, 8–10 per cent (more than eight minutes after inhalation); P, 6 mg per kg (within seven minutes of intravenous injection). Deep stage: H, 2–3 per cent (more than five minutes after inhalation); E, 15–20 per cent (more than eight minutes after inhalation); P, 10–20 mg per kg (within seven minutes of intravenous injection).

Units which showed changes in waveforms during the observation were excluded from analysis. Only after confirmation of full recovery of action potentials from an anesthetic was the administration of another anesthetic started. The interval between inhalation of two different agents was usually two to three hours. Depth of anesthesia and recovery were judged by several reflex activities and ongoing EEG patterns.

The site of the electrode tip was identified histologically. At the termination of a recording period, dc current was applied to the microelectrode (10–20 μA for tungsten and 2 μA for stainless steel for about 30 seconds[7,8]). When stainless steel electrodes were used, potassium ferrocyanide, 1 per cent, was injected through the carotid artery of the recording side before perfusion of the brain with 10 per cent formalin solution. Identification of the entire track of the microelectrode was achieved by fixing the brain with formalin with the electrode in place. After fixation for a week, the brain was cut along the entire microelectrode track (fig. 2). The discrepancies between electrode positions as identified by the microdriver scale and as determined by histologic examination were minimal.

Results

Of 83 units tested, 37 were analyzed. The rest were excluded from analysis because changes in waveforms or amplitudes of unitary spikes were found during the hour allowed for attainment of stability.

TABLE 1. Mean Firing Rates of MRF Units during wakefulness and Three Stages of Anesthesia with Four Anesthetics*

Unit	Awake	Halothane Anesthesia		
		Light	Moderate	Deep
9–22–1	7.3	2.2	0	0
9–22–2†	33.2	15.7	18.1	0
9–22–3	6.7		4.1	
9–22–4	1.4		*3.3*	0
9–22–5†	4.9		1.2	
10– 3–1	1.0		0	
10– 3–4	1.2		0	
10–13–1	19.3		*10.4*	3.1
10–13–2	12.5		0.8	
10–13–3	8.0		*12.0*	0
10–24–1	1.8		0.7	
10–24–2	6.3		0.5	
10–20–1†	11.2		5.3	
10–26–3	11.6		2.6	
11–10–2†	9.3		2.1	
11–10–3†	3.3		0.7	
11–10–4†	4.1		0.4	
11–10–5†	5.2		0.4	0
11–22–3†	8.4	0.9	1.2	0
11–29–1†	14.2	8.3	1.8	
11–29–2	12.5	8.6	4.7	0
11–29–4†	12.8		0.3	
11–29–5	2.4	1.1	0.8	0
11–29–6†	8.0		0.7	
12– 8–1†	24.5	28.0	7.9	
1–13–2†	12.6		*32.0*	20.7
1–30–2†	10.6		0	
1–30–3†	22.6		21.0	8.4
1–30–4†	33.9		6.5	
2–27–4†	12.9		5.7	

Unit	Awake	Diethyl Ether Anesthesia		
		Light	Moderate	Deep
9–22–2†	33.2	27.8	25.0	0
9–22–5†	4.9		3.5	
10– 3–3	0.7		0	
10–26–1†	11.2		*18.3*	3.7
11–10–2†	9.3		0	
11–10–3†	3.3		0	
11–10–4†	4.1		0.1	
11–20–3†	8.4	2.8	2.0	0
11–29–1†	14.2	12.5		
11–29–4†	12.8		1.2	
11–29–6†	8.0		0	
12– 8–1†	24.5	31.8	16.2	1.8
1–13–2†	12.6		11.3	
1–30–2†	10.6		0	
1–30–3†	22.6		20.7	
1–30–4†	33.9		7.2	
2–27–4†	12.9		0.6	

Unit	Awake	Sodium Thiopental Anesthesia		
		Light	Moderate	Deep
11–10–4†	4.1	0		
11–10–5†	5.2	0		
11–22–1†	7.7	0.4		
11–29–1†	14.2	4.4		
11–29–4	12.8		0	
12– 8–1†	24.5		2.5	
1–30–4†	33.9		5.6	0
2–27–4†	12.9		8.1	2.3

Unit	Awake	Nitrous Oxide Anesthesia		
		Light	Moderate	Deep
11–22–1†	7.7	15.6		
11–22–3†	8.4	0.7		
2–20–1	24.7	11.9		
12– 8–1†	24.5	26.7		
1–30–1	22.1	18.5		
1–30–2†	10.6	6.1		
2–27–1	7.4	16.8		
2–27–2	5.1	0.9		
2–27–3	0	9.4		
2–27–4†	12.9	10.1		

* Computation was made after 30 seconds at each stable state. Mean firing rates in each anesthetic state are significantly different ($P < 0.01$) from those of wakefulness in all units. Italicized numbers indicate increases in firing rates at the moderate stage of anesthesia.

† More than one anesthetic was studied.

SUSCEPTIBILITY OF UNITS TO ANESTHETICS

The mean rates of firing per second during the unanesthetized and anesthetized state were computed and are summarized in table 1.

During the unanesthetized state, all units tested were spontaneously active except one (table 1, unit 2–27–3), which gained its spontaneous activity during the light stage of nitrous oxide anesthesia.

In most units (32/37), spontaneous firing rates decreased as anesthesia progressed. However, there were also units (5/37) in which spontaneous firing rates increased during the moderate stage of anesthesia. In the deep stage of anesthesia with each anesthetic, spon-

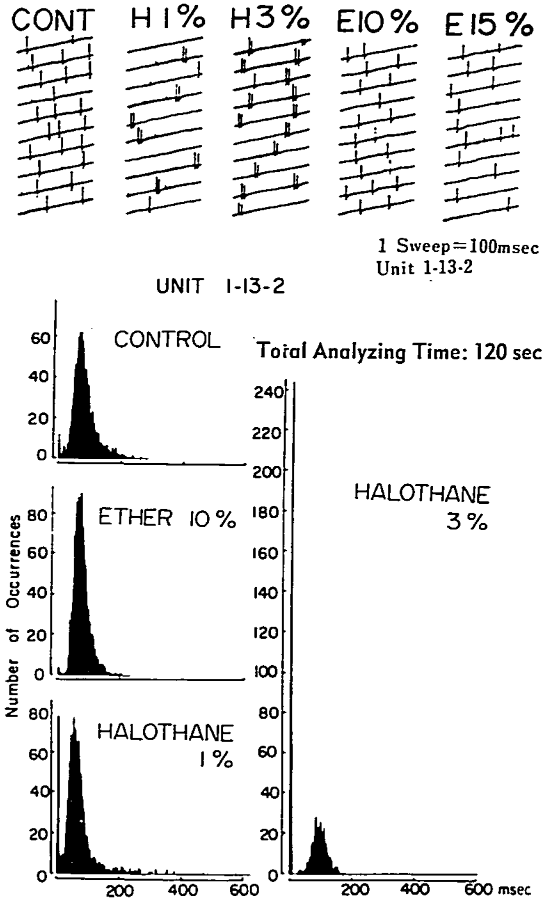


Fig. 3. Firing patterns of one MRF unit. The characteristic "grouped" firing is evident during halothane anesthesia (H 1% and H 3%). The same unit fired in a "tonic" fashion during diethyl ether anesthesia (E 10% and E 15%). A (*above*), oscilloscopic traces. B (*below*), interval histogram of the same unit.

taneous activity decreased or ceased in all units except one (1–13–2), in which the firing rate increased considerably even during the deep stage of anesthesia. Susceptibility of the units to anesthetic drugs varied widely (table 1). In general, though, a unit highly susceptible to one anesthetic was also sensitive to the others.

Significant initial transient increases in firing rates were observed in most instances of diethyl ether inhalation (15/17) and sometimes during halothane inhalation (6/29), but not with either sodium thiopental or nitrous oxide anesthesia.

Spontaneous Firing Patterns

During anesthesia two patterns of firing, "grouping" and "tonic discharges," were ob-

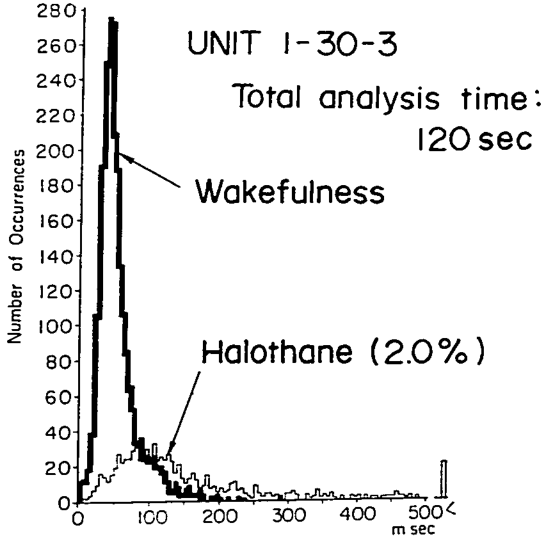


FIG. 4. Two interval histograms of one MRF unit during wakefulness and a deep stage of halothane anesthesia. In this unit the firing rate decreased in a "tonic" fashion during halothane anesthesia. Numbers of spike intervals: 2,354 during wakefulness; 845 during halothane anesthesia. Spike intervals: 52.2 ± 31.6 msec (mean ± SD) during wakefulness; 149.2 ± 123.5 msec during halothane anesthesia.

served. The occurrences of these two firing patterns of MRF neurons during wakefulness and on exposure to four anesthetics are summarized in table 2. "Grouped" firing was more common during sodium thiopental and halothane anesthesia; tonic firing was seen more frequently during diethyl ether and nitrous oxide anesthesia.

Figure 3 shows an example of two firing patterns elicited in one unit by two anesthetics. It is evident in IH (fig. 3*B*) that the shortest interspike intervals increase in halothane anesthesia, while in diethyl ether anesthesia such a pattern does not occur.

The tendency to fire in "groups," seldom observed during the unanesthetized or wakeful state, became more predominant as anesthesia deepened (fig. 3). That is, units increasingly fired in "groups," with prolongation of the intervening periods of silence as anesthesia deepened.

The rest of the units showed another pattern. They gradually decreased "tonically" their spontaneous firing rates, with prolongation of sequential interspike intervals as anesthesia progressed (fig. 4).

Correlation of MRF Unit Firings with the Corticogram

No correlation between spontaneous MRF unit firings and the electrocorticogram during the unanesthetized state could be identified. In two units, however, synchronization between MRF unit firings and positive sharp waves of the corticogram was clearly apparent during the deep stage of sodium thiopental anesthesia. This was proved more conclusively by averaging the cortical activity with reference to unit firing (fig. 5). It is evident that the MRF unit activity is associated with the cortical wave configuration of a sharp posi-

TABLE 2. Classification of MRF Units According to Spontaneous Firing Patterns during Wakefulness and on Exposure to Four Anesthetics

	Awake	N_2O	Ether	Halothane	Thiopental
"Grouped"	0	3	1	17	8
Tonic	37	7	16	12	0
TOTAL	37	10	17	29	8

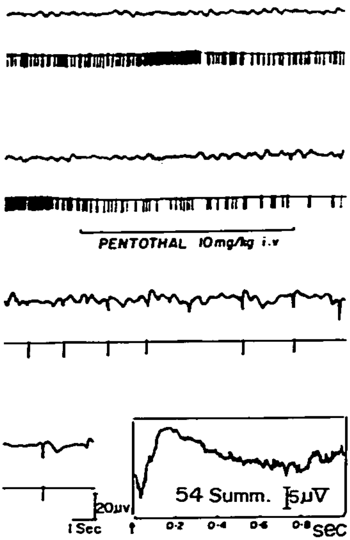


FIG. 5. Simultaneous recording of the corticogram and standard pulses triggered by the unit firings. Note the synchronization between the sharp positive waves of the corticogram and unit discharges after the administration of sodium thiopental. A cortical wave averaged with reference to unit firing is shown at bottom right.

tive wave about 100 msec in duration followed by a slow negative wave lasting more than 0.5 sec.

Discussion

In this study, "spontaneous activity" of units means that during the quiet awake or anesthetized state, at constant ventilatory volume, with the cat fixed gently in the stereotaxic apparatus and free from noxious stimuli.

The spontaneous firing rates of MRF units during quiet wakefulness in the present study ranged from 0 to 33.9 per second. This value is smaller than that in chronic cats reported by Huttenlocher.[5] The difference may be due to the smaller input impulses from joint and muscle receptors in the present experiment.

Our data indicate that, unlike natural sleep, adequate doses of anesthetics have a depressant effect upon spontaneous activity of the MRF. Susceptibility to anesthetics, however, varies from unit to unit.

Periodic fluctuations in impulse frequency for a time period determined by calculation of FC were also observed in all MRF units during both unanesthetized and anesthetized states. Efforts to elucidate the difference between these periodic fluctuations of impulse frequency in unanesthetized and anesthetized states yielded no consistent finding.

Factors causing differences in neuronal activity in response to anesthetics may be divided into the effect of an anesthetic itself and the epiphenomena, or side-effects. For example, changes in the arterial blood pressure induced by anesthetics could modify the activity of MRF units.[9] The greatest change in arterial blood pressure occurred within 10 minutes of the administration of an anesthetic in present study.

Thereafter, arterial blood pressure remained relatively constant. The average systolic pressures during moderate stages of halothane, ether, and thiopental anesthesia were 79, 94, and 86 per cent of control, respectively. However, the responses of MRF units to anesthetics were slower than the changes in arterial pressure and were variable from unit to unit. The author could not find any definite correlation between unitary behavior in the MRF and changes in arterial blood pressure. Review of the literature[10–19] reveals that it is not necessarily assumed that the differential effects of anesthetics upon the spontaneous activities of MRF units are due to their side-effects.

The "grouped firing" of MRF units, more characteristic of sodium thiopental and halothane anesthesia than diethyl ether and nitrous oxide anesthesia, was also observed during natural sleep with EEG slow waves by Huttenlocher.[5]

It should be possible to identify a correlation between patterns of cortical waves and MRF unit firings, since several pieces of evidence for the existence of reticulocortical or corticoreticular connections have been accumulated.[1,2] Unfortunately, any correlation between each unitary spike of the MRF and the cortical wave was seldom recognizable during wakefulness. The synchronization between MRF unit firings and cortical positive sharp waves observed during deep sodium thiopental anesthesia may support the hypothesis of synchronization of neuronal activity during anesthesia.[20] However, the mechanism by which the synchronization of the positive sharp cortical waves with MRF unit firings became unmasked during the deep stage of sodium thiopental anesthesia was not elucidated in the present study.

The authors appreciate the criticism of this study of Professor K. Iwama. The authors also thank Dr. J. M. Messick, Jr., for assistance in preparation of the manuscript.

References

1. Moruzzi G, Magoun HW: Brain stem reticular formation and activation of the EEG. Electroenceph Clin Neurophysiol 1:455–473, 1949
2. French JC, Verzeano M, Magoun HW: A neuronal basis of the anesthetic state. AMA Arch Neurol Psychiat 69:519–529, 1953
3. Arduini A, Arduini MG: Effect of drugs and metabolic alterations on brain stem arousal mechanism. J Pharmacol Exp Ther 110: 76–85, 1954
4. Forbes A, Morison BR: Cortical response to sensory stimulation under deep barbiturate narcosis. J Neurophysiol 2:112–128, 1939
5. Huttenlocher PR: Evoked and spontaneous activity in single units of medial brain stem during natural sleep and waking. J Neurophysiol 24:451–468, 1961
6. Amassian VE, Macy J, Waller HJ: Patterns of activity of simultaneously recorded neurons in midbrain reticular formation. Ann NY Acad Sci 89:883–895, 1961
7. Hubel DJ: Tungsten microelectrode for recording from single units. Science 125: 549–550, 1957
8. Green JD: A simple microelectrode for recording from the central nervous system. Nature 182:962, 1958
9. Baust W, Niemczyk H: Studies on the adrenaline-sensitive component of the mesencephalic reticular formation. J Neurophysiol 26:692–704, 1963
10. Eccles JC, Schmidt R, Willis WD: Pharmacological studies on presynaptic inhibition. J Physiol 168:500–530, 1963
11. Somjen GG, Gill M: The mechanism of the blockade of synaptic transmission in the mammalian spinal cord by diethyl ether and by thiopental. J Pharmacol Exp Ther 140: 19–30, 1963
12. Somjen GG: Effects of ether and thiopental on spinal presynaptic terminals. J Pharmacol Exp Ther 140:396–402, 1963
13. Løyning Y, Oshima T, Yokota T: Site of action of thiamylal sodium on the monosynaptic spinal reflex pathway in cats. J Neurophysiol 27:408–428, 1964
14. Miyahara JT, Esplin DW, Zablocka B: Differential effects of depressant drugs on presynaptic inhibition. J Pharmacol Exp Ther 154:118–127, 1966
15. de Jong RH, Robles R, Heavner JE: Suppression of impulse transmission in the cat's dorsal horn by inhalation anesthetics. ANESTHESIOLOGY 32:440–445, 1970
16. Rosenberg P, Bartels E: Drug effects on the spontaneous electrical activity of the squid giant axon. J Pharmacol Exp Ther 155: 532–544, 1967
17. King EE: Differential action of anesthetics and interneuron depressants upon EEG arousal and recruiting responses. J Pharmacol Exp Ther 116:404–417, 1956
18. Galindo A: Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. J Pharmacol Exp Ther 169:185–195, 1969
19. Shimoji K, Bickford RG: Firing patterns of mesencephalic reticular neurons related to a variety of anesthetic agents (abstract). Fed Proc 26:763, 1967
20. Faulconer A, Bickford RG: Electroencephalography in Anesthesiology. Springfield, Charles C Thomas, 1960, pp 12–18

Drugs

DEATH FROM ASPIRIN A 23-year-old woman died following the ingestion of aspirin. Since large amounts of aspirin tablets in the gut seem to retard their own absorption, it is essential that serial salicylate determinations be obtained following the ingestion to make certain that continued increases in serum salicylate levels do not occur. Serum salicylate concentrations did not correlate with the state of consciousness or the magnitude of metabolic disturbance. The usefulness of gastric lavage in removing undissolved tablets is questioned. *(Ferguson, R. K., and Boutros, A. R.: Death Following Self-poisoning with Aspirin, J.A.M.A. 213: 1186 (Aug.) 1970.)*