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Electron Microscopy of the Macaca mulatta *Brain after Repeated Applications of Electric Current*

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Portions of the brains of three Rhesus monkeys were examined by electron microscopy. No current was applied to the brain of the first monkey. The second and third each received ten hours of application of electric current during a five-week period, one hour per application. One monkey received variations of sine current only, the other, variations of square-wave current only. Sections from the brains of all three were examined by light and electron microscopy. No changes attributable to the application of electric current were found. (Key words: Brain; Electric current; Electroanesthesia.)

THIS STUDY was part of a continuing inquiry into the safety of the application of electric current for purposes of electroanesthesia. In particular, the study was designed to investigate the subcellular or ultrastructural effects in the brain of the Rhesus monkey of repeated applications of current. Previously, Smith *et al.*[1] studied the brains of eight dogs grossly and by light microscopy after various periods of electronarcosis and found no changes attributable to the passage of electric current. Later, Smith *et al.*[2] examined three dogs' brains in more detail by light and electron microscopy. They demonstrated that repeated electroconvulsive therapy and electroanesthesia caused no gross, microscopic, or ultrastructural lesions in the brains of these animals. The present study was designed to investigate the permanent effects, if any, of two basic forms of electroanesthetic currents on the ultrastructure of the brain in the primate.

Methods

The experimental work was carried out in the Department of Anesthesia at the University of California Medical Center in San Francisco, and then the live monkeys were flown to North Chicago for examination.

Three immature Rhesus monkeys (weights 2.5–3.5 kg) were used for study. Monkey 1, the control animal, was not subjected to application of electric current. The second and third monkeys each received ten hours of application of electric current during a five-week period, twice each week, one hour per application. Monkey 2 received variations of pulse current only; Monkey 3, variations of sine current only. (See tables 1 and 2 for summaries of current applied, instruments used, numbers of electrodes, and positions of electrodes). Saline-soaked sponge electrodes with palladium plate and lead wire were used in all applications; electrodes were secured with adhesive tape.

The instruments used and their designations were: HP 200 CD, Hewlett-Packard Model 200CD, wide-range oscillator; modified for electric motor drive with variable speed; HP 3380B, Hewlett-Packard electroanesthesia unit; EMI 100A, experimental square-wave, constant-current generator.

Before each application of current, a naso-

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TABLE 1. Monkey 2. Applications of Pulsatile Current from a Constant-Current Square-Pulse Experimental Electroanesthesia Generator

Trial	Electrode Positions	Pulse Polarity	Switching Mode*	Switching Rate (cps)†	Frequency‡	Duty Cycle§	Maximum Milliamperage Attained	Anesthesia¶	Fading
1	Bitemporal	Unidirectional positive	C-A	170	Variable 365–600	22 per cent	11	2	+
2	Bitemporal	Unidirectional positive	C-A	146	Variable 320–500	22 per cent	10	1–2	+
3	Bitemporal	Unidirectional positive	C-A	175	Variable 750–1,500	22 per cent	13	1	+
4	Bitemporal; fronto-occipital	Undirectional, positive	C-A	155	Variable 900–1,100	22 per cent	11	1	+
5	Bitemporal; fronto-occipital	Bidirectional, alternating positive and negative	C-A	160	Variable 760–900	30 per cent	11	1	+
6	Bitemporal; fronto-occipital	Bidirectional, alternating positive and negative	C-A	148	Variable 1,960–2,500	40 per cent	10	1–2	+
	Bitemporal; fronto-occipital	Bidirectional, alternating positive and negative	C-A	140	Variable 490–660	44 per cent	9	1–2	+
8	Bitemporal	Bidirectional, alternating positive and negative	Manual	Variable 88–1,000	1,000	40 per cent	7	1–2	+
9	Bitemporal	Bidirectional alternating positive and negative	Fixed frequency	2,000	Variable 105–175	43 per cent	11	1–2	+
10	Bitemporal; fronto-occipital	Bidirectional, alternating positive and negative	Fixed frequency	2,000	4,000	70 per cent	10	1–2	+

* C-A = Charge-adding switching; polarity of electrodes changed electronically at a rate determined by number of millicoulombs passed through each electrode.
† Switching frequency linearly proportional to current level.
‡ Variable frequency with HP 200CD motor-driven oscillator (1 complete cycle/30 sec).
§ Percentage of pulse "on" time to complete cycle.
¶ See text for grading of anesthesia.

tracheal catheter was passed and secured. Ventilation was effected with an Ambu bag and room air whenever a test animal held its breath during induction. Anesthesia was defined as lack of body movement in response to a square-wave stimulus passed through two 22-gauge needles in the tail.

Anesthesia was graded 0–4 by these criteria: 1, poor anesthesia, marked muscle activity; 2, fair anesthesia, moderate muscle activity; 3, good anesthesia, moderate muscle activity; 4, excellent anesthesia, quiet.

Within 48 hours of the last application of current, the monkeys were sacrificed with a lethal dose of pentobarbital sodium injected intravenously and the brains were removed immediately. No more than ten minutes passed from the time of death until fixation for electron microscopy had been completed. Specimens from 13 areas of each brain were collected, as follows: four separate parts of the cerebral cortex; corpus callosum; caudate nucleus; internal capsule; thalamus; hypothalamus; pons; medulla; cerebellum; superior colliculus.

The tissues for light microscopy were fixed in 10 per cent formalin and processed in a routine manner, sectioned, and stained. The tissues for electron microscopy were fixed by mincing in cold osmium tetroxide buffered with veronal acetate and with sucrose added. Small bits of tissue, 2 to 3 mm square, were dehydrated and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate

TABLE 2. Monkey 3. Applications of Sine Current

Trial	HP 3380B		Electrode Positions†	Maximum Milli-amperage Attained‡	Anesthesia§	Fading	EMI 100A Frequency (Kc)
	High-frequency (Kc)	Low-frequency (Kc)*					
1	0.80 1.0 1.5 2.0	15 per cent; 10 per cent Variable 0.60–0.90	Bitemporal	30	1	+	
2	3 3.5	10 per cent; 20 per cent Variable 0.8–1	Bitemporal	40	1–2	+	
3	3 3.6 4.0	10 per cent Variable 0.52–0.66	Bitemporal	40	1	+	
4	3.4 4.0 2.2 2.0	20 per cent Variable 0.90–1.10	Bitemporal	50	1–2	+	
5	0.98 1.3	0	Bitemporal; fronto-occipital	25; 25	2–3	+	0.9–1.1 Variable
6	2.6 1.8	0	Bitemporal; fronto-occipital	25; 25	1–2	+	1.96–2.5 Variable
7	1.3	0	Bitemporal; fronto-occipital	20; 20	1	+	0.92–1.1 Variable
8	0.72	10 per cent	Bitemporal; fronto-occipital	15; 15	1	+	0.70 Fixed
9	2	0	Bitemporal; fronto-occipital	25; 25	1–2	+	2.2–3.5 Variable
10	0.70 0.73	0	Bitemporal; fronto-occipital	15; 15	1	+	0.67–0.72 Variable

* Frequency varied with HP 200CD motor-driven oscillator. One complete cycle/30 sec.
† For two pairs of electrodes, EMI 100A was applied to the fronto-occipital pair.
‡ For two pairs of electrodes, maximum ma for each pair is given.
§ See text for grading of anesthesia.

and lead citrate. Sections cut at 1 μ were stained with toluidine blue for oil-immersion light microscopy.

To compensate for the sampling problem inherent in electron microscopy, we cut large numbers of sections and also prepared larger 1-μ sections for study by oil-immersion light microscopy. Two blocks of each specimen were cut, and three grids were prepared from each block. Thus, six grids were prepared from each of the 13 areas of brains, for a total of 78 grids from each monkey. Each grid contained several ribbons of sections. All grids were examined independently as unknowns by two people. More than 300 micrographs were prepared for detailed study.

Results

This study afforded an opportunity to investigate several variations of sine and pulsatile current applications in a effort to produce satisfactory, sustainable anesthesia in the Rhesus monkey. The results of this effort are summarized in tables 1 and 2. In general, anesthesia was only poor-to-fair with all variations used; fading or arousal from anesthesia was seen in every one of the 20 trials.

The gross histologic and ultrastructural examination of the brains of the three monkeys did not reveal any evidence of injury. There were no structural variations among the three animals, one of which was used as a control. All samples of tissue were well fixed, and few artifacts of fixation were identified. The last areas of brain sampled were essentially as well fixed as the first areas sampled. Thus, the few minutes needed to complete the fixation of tissue did not result in noticeable fixation artifact. The only changes seen were reduction in contrast and reduced staining of the membrane structures in the last tissues to be fixed and occasional mitochondrial swelling in all animals.

In examining the tissues, the following components were identified and examined individually: neurons; neuroglia; myelinated and nonmyelinated fibers; capillaries and nearby astrocytes; synaptic endings and the general architecture of the neurophil. Special attention was given to the structure of the synapse and the size, location, and distribution of synaptic vesicles. Counts were not made of vesicles in the presynaptic region, but there was no noticeable change in their numbers. All of these structures were within normal limits.

Discussion

Previous ultrastructural examinations of dog brain revealed no lesions associated with electroanesthesia. These findings are supported by our similar findings in monkeys. Negative studies such as this are not conclusive, because a random lesion could be missed with the sampling problem inherent in electron microscopy. However, we can say that there is no generalized evidence of injury, and we have greatly increased our confidence in the safety of repeated application of these electric currents.

In reviewing reports of electroshock, Meyer[3] indicated that lesions may occur following repeated electroshock, but that they are not constant in all individuals and are of a minimal or isolated nature. These lesions, consisting of degenerated neurons and slight gliosis, could have been secondarily related to anoxia or could have resulted from unrelated causes. Also, we do not know what effect higher current density might produce.

Siegesmund *et al.*[4] described changes in the numbers of synaptic vesicles during application of current. The numbers returned to normal soon after the current was turned off.

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