Effect of Section of the Corpus Callosum on Cortical After-Discharge Patterns in the Cat.* (32031)

ROBERT N. STRAW† AND C. L. MITCHELL
Department of Pharmacology, College of Medicine, University of Iowa, Iowa City

Hoefer and Pool(1) have reported that after section of the corpus callosum in the cat, cortical after-discharge could still be recorded from the contralateral hemisphere. These investigators stimulated the motor cortex, an area with a relatively high seizure threshold in this species(2).

In contrast, section of the corpus callosum has been reported to abolish the cortical after-discharge in the hemisphere contralateral to that stimulated when the after-discharge response was elicited by stimulation of the motor cortex in the monkey(3). However, Poblete *et al*(4) reported that section of the corpus callosum has no effect on after-discharge patterns elicited by stimulation of the temporal pole in the monkey. These two areas of the monkey cortex (*i.e.*, motor and temporal) have a relatively low and relatively high seizure threshold, respectively(5).

Garner and French(2) have attributed the difference in the seizure threshold of the motor cortex between the two species to the degree of representation of that area in the corpus callosum. The monkey has a relatively high percentage of callosal fibers arising in the motor cortex whereas, in the cat, a relatively small percentage arises in this area (6). Further, the cat has a relatively high percentage of callosal fibers arising in the ectosylvian gyrus(6), a cortical area with a relatively low seizure threshold(2).

In view of the reports that(1) section of the corpus callosum will block the interhemispheral spread of after-discharge when an area with a relatively high degree of representation is stimulated in the monkey and (2) corpus callosum section will not affect the after-discharge patterns when an area with a relatively low degree of representation is stimulated in the monkey and cat, it was of interest to evaluate the effect of section of the corpus callosum on after-discharge patterns elicited by stimulation of the ectosylvian cortex in the cat, a region with a relatively high representation in the corpus callosum.

Methods. Twelve adult cats of both sexes were used in these experiments. The animals were surgically prepared under ether anesthesia. The brachial vein was cannulated for all drug injections. The femoral artery was cannulated and the arterial pressure was monitored *via* a Statham P 23 transducer and recorded on a Gilson Polygraph. The rectal temperature was monitored by means of a local thermistor *via* a Yellow Spring telethermometer. Body temperature was maintained between 36° and 38°C.

Steel phonograph needles were tapped into the skull for recording the EEG. Stainless steel screws with a flat tip (approximately 1.5 mm in diameter) were threaded into the skull to serve as stimulating electrodes.

Following the completion of surgery the animals were immobilized with gallamine triethiodide (Flaxedil) and respired by means of a Harvard pump set at 16 strokes/min. Additional doses of gallamine triethiodide were given every 40 minutes. At least a 2 hour interval elapsed between the discontinuance of the ether and the beginning of the experiment. All open wound edges and pressure points were infiltrated with 0.05% dibucaine (with epinephrine).

The area of the cortex stimulated was the medial ectosylvian gyrus of the right hemisphere. The holes for the stimulating electrodes were stereotactically located (coordinates: A10, L16 and A2, L14; (7). Bipolar EEG recordings were made bilaterally *via* electrodes on the posterior sigmoidal and posterior lateral gyri. In addition, bipolar recordings were made from the medial ectosylvian gyrus of the left hemisphere. All EEG recordings were made on either an Offner type T or Grass model III EEG.

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TABLE I. Effect of Section of the Corpus Callosum or Sham Procedure on Parameters of After-Discharge in the Acute Preparations.

Hours after procedure	Seizure threshold*		After-discharge duration†	
	Sham	Section	Sham	Section
Control	8 ± 2.4	7 ± .7	24 ± 8.8	95 ± 37.9
1	8 ± 2.6	6 ±1.0	12 ± .6	64 ± 30.8
2	8 ± 2.7	6 ± .6	18 ± 4.9	76 ± 27.3
3	8 ± 2.8	6 ± .6	53 ±25.3	106 ± 55.7

* Values are mean volts ± standard error. None of the threshold values determined at any time after the procedure (*i.e.*, sham or section) were significantly different from their respective control value (Student's t, paired comparison, $p > 0.05$). There were 3 animals in each group.

† Values are mean duration (sec) ± standard error. None of the durations recorded at any time after the procedure were significantly different from their respective control value (Student's t, paired comparison, $p > 0.05$).

In all experiments, a 5 second train of 1 msec monophasic pulses was delivered from a waveform (Tektronix type 162) and pulse (Tektronix type 161) generator at 50 pulses per second and at a threshold voltage. To determine this voltage, an initial voltage of 2 V was used. The voltage was then increased by 1 V increments until a bilateral after-discharge was elicited. At least 5 minutes elapsed between stimuli. Three such threshold determinations were made at 15 minute intervals, The starting voltage for the second and third trials was 2 V below that determined in the first threshold trial.

Following 3 control determinations, the corpus callosum was sectioned (in 3 cats) using the method described by Magni *et al* (8). In other animals (3 cats), a sham procedure was used. The dura was incised and reflected as before and the needle tubing was lowered approximately 5 mm into the medial longitudinal fissure but the string used to section the corpus callosum was not passed through the tubing.

Following the section of the corpus callosum or sham procedure, threshold determinations were made as described above at 1, 2 and 3 h following the procedure.

In the remaining animals, the section of the corpus callosum or sham procedure was performed under pentobarbital sodium anethesia (25 mg/kg). The skull opening was covered with Gelfoam and bonewax and sealed with dental acrylic which was anchored to the skull by means of 4 small stainless steel screws. Ten to 14 days following the surgery, the animals were prepared as described above. The experimental procedure was exactly the same as previously described except that no surgical manipulation (*i.e.*, section or sham) was performed during the course of the experiment.

At the close of each experiment the brain was removed and placed in 10% formalin in normal saline. After it was fixed, it was sliced in approximately 2 mm coronal sections to determine the extent of the section of the corpus callosum and the degree of involvement of other structures.

For purposes of statistical comparisons, the data of the 3 control trials were grouped to form a period. Similar groupings were made for each of the other time periods and for all trials in those experiments in which the animals were chonically prepared. Statistical comparisons were made by means of a Student's t test.

Results. Section of the corpus callosum. The method employed in this investigation yielded complete section of the corpus callosum in all 6 animals in which such a section was attempted. In addition, there was varying degrees of involvement of the body of the fornix. This ranged from only minimal sectioning at its most rostral extent to almost total section of this structure. The extent to which the fornix was involved did not appear to influence the parameters of after-discharge to be described. In the 6 sham animals, there was no involvement (*i.e.*, damage) to any midline structure.

Parameters of after-discharge. Table I lists the mean threshold values and mean duration of after-discharge values determined in the control period and at the 3 time intervals

TABLE II. Parameters of After-Discharge in Animals Surgically Prepared 10 to 14 Days Prior to the Experiment.

Period	Seizure threshold*		After-discharge duration†	
	Sham	Section	Sham	Section
1	9 ± 3.5	6 ± 1.3	24 ± 2.5	18 ± 6.6
2	10 ± 3.7	6 ± 1.3	58 ± 33.2	95 ± 39.9
3	12 ± 4.7	6 ± .9	86 ± 19.7	57 ± 28.4
4	10 ± 3.7	5 ± .6	110 ± 53.4	71 ± 33.7

* Values are mean volts ± standard error. In no case was the mean threshold of those animals whose corpus callosum was sectioned different from that of those animals who had the sham procedure (Student's t, $p > 0.05$). There were 3 animals in each group.

† Values are mean duration (sec) ± standard error. In no case was the mean duration of those animals whose corpus callosum was sectioned different from that of those animals who had the sham procedure (Student's t, $p > 0.05$).

after section of the corpus callosum or sham procedure during the experiment. As can be seen there were no significant differences in either parameter between the control values and that determined at any of the times examined in either the section or sham group of animals.

Table II lists the mean values of the seizure threshold and after-discharge duration for the 2 groups of animals which were surgically prepared 10 to 14 days prior to the experiment. The apparent difference in the threshold between the sham and section groups of animals is not significant at any of the periods. In 2 of the 3 cats in the sham group, the threshold value was similar to that of the section group. However, in the remaining cat the threshold was somewhat elevated, thus accounting for the higher mean value of the sham group.

In addition, there was no significant difference in the after-discharge duration between the 2 groups of cats. Further, the apparent increase in the seizure duration over the trials is not significant in either the sham or section group, due to variability among animals.

The duration of after-discharge in all cats, regardless of the procedure (*i.e.*, sham or section) or the time of the procedure (*i.e.*, during the experiment or 10 to 14 days prior to the experiment), varied inconsistently from trial to trial. This variability was of the magnitude which we have previously described for cats with no surgical manipulation of the corpus callosum(10).

EEG patterns. There were no consistent differences between the EEG recorded after either section of the corpus callosum or the sham procedure, and that recorded during the control period. In 2 (1 sham, 1 section) of the animals, the voltage at the height of the after-discharge appeared to be somewhat depressed after the operative procedure. There were also no consistent differences in the EEG patterns of the animals that were surgically prepared 10 to 14 days prior to the experiment.

An example of the EEG patterns recorded prior to and after section of the corpus callosum is shown in Fig. 1.

Discussion. These results, together with those of Hoefer and Pool(1), suggest that the corpus callosum is not the primary pathway for the spread of cortical after-discharge to the contralateral hemisphere when it is elicited by stimulation of either the motor or ectosylvian cortex in the cat. This is in contrast to the monkey in which the after-discharge elicited by stimulation of the motor cortex has been reported to spread *via* the corpus callosum(3). However, that elicited by stimulation of the temporal pole does not appear to spread *via* the corpus callosum but rather *via* the anterior commissure(4). The temporal gyri of the monkey may be thought of as being comparable to the ectosylvian cortex of the cat, at least with respect to epileptogenic tendencies(2).

Since Garner and French(2) have stated that the seizure threshold of a cortical area is at least in part due to the contribution of that area to the corpus callosum, one might expect that the spread of the after-discharge response elicited by stimulation of the ectosylvian cortex would be blocked by section of the corpus callosum. However, as the data

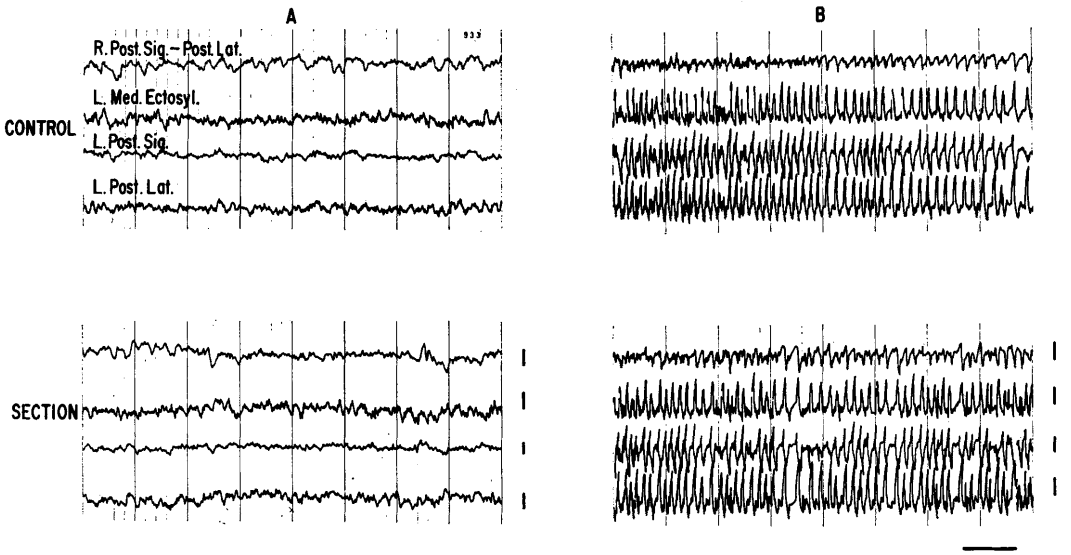

FIG. 1. Example of after-discharge patterns recorded from a cat in which the corpus callosum was sectioned during the experiment. A = control bioelectrical activity; B = height of after-discharge. The lower tracings were recorded approximately 2 hr after section of the corpus callosum. The horizontal time line = 2 sec; the vertical calibration bars for panel A (in both control and section) = 50 μV; for panel B (in both control and section) = 250 μV.

presented herein clearly show, such is not the case.

No significant differences in the threshold between the sham and section group of animals were found in this investigation. However, there was a tendency for the threshold to be lower in the section than in the sham group of cats. This is in contrast to the report of Hoefer and Pool(1) who found an increase in the seizure threshold after section of the corpus callosum. However, these investigators made no attempt to quantitate their data. Nevertheless, the significance of this investigation bears not upon possible threshold alterations but rather upon the fact that a bilaterally distributed cortical after-discharge was always observed after-section of the corpus callosum.

Thus it would appear that other subcortical midline structures play the major role of interhemispheral spread of cortical after-discharge in the cat. It would seem that the body of the fornix does not play a major role in the cat as this structure was sectioned to varying degrees in this investigation with no apparent effect on the after-discharge patterns. Therefore, it would appear that other structures (*e.g.*, anterior commissure) are probably the major pathway(s).

In contrast, there seems to be little doubt as to the necessity of an intact corpus callosum for the formation of a secondary focus in either the cat or monkey (c.f. 11, 12). Thus, it would seem that further investigation in the cat of the differences between the after-discharge response and the formation of a secondary epileptogenic focus may lead to a better understanding of some of the pathophysiological processes occurring in epilepsy.

Summary. The effect of section of the corpus callosum on cortical after-discharge patterns elicited by stimulation of the ectosylvian cortex in the cat was examined. Such a procedure had no apparent effect on any parameter of cortical after-discharge examined.

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Immunologic Restoration of Neonatally Thymectomized Rats With Thoracic Duct Lymphocytes. (32032)

HEWES D. AGNEW (Introduced by L. W. Law)
Laboratory of Biology, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Previous investigations have established that neonatal thymectomy in the rat, as with other rodents, leads to immunologic deficits. While the wasting syndrome that routinely follows neonatal thymectomy in many strains of mice seems to be less common (1), neonatal thymectomy in the rat is characterized by a depletion of small lymphocytes from blood and lymphoid tissue(2), absence or depression of delayed hypersensitivity and Arthus reaction, and a decrease in production of some but not all humoral antibodies(3,4). There is also an increased survival of skin homografts when animals of minimal genetic disparity are employed as donor and host(5). Furthermore, an increased susceptibility to the induction of tumors by polyoma virus and murine sarcoma virus (Moloney strain) has been noted(6,7).

Experiments in which the neonatally thymectomized animal is "restored" with either an injection of a certain cell type or graft of a specific organ have been carried out to ascertain information concerning both the nature of the deficit of the thymectomized animal and the function of donor tissue. Restoration experiments have shown that the immunologic deficit incurred following thymectomy can be reversed either by restoring to the animal the factors necessary for its own immune system to mature to a state of immunological competence or by re-equipping the animal with immunologically competent cells taken from normal adult syngeneic donors.

The latter type of experiment in which thymectomized animals have been re-equipped or reconstituted with immunologically competent donor lymphoid tissue have been performed using either spleen or lymph nodes as the source of cells. Because of the heterogeneous population of cells in the spleen and lymph node it has not been possible to say with exactness the actual cell involved in these restoration experiments. Cannulation of the thoracic duct of the rat yields a morphologically relatively homogeneous population of cells, consisting of greater than 95% small lymphocytes and the residual large lymphocytes(15). It seemed of interest, therefore, to test the ability of thoracic duct cells to restore the immunological deficits of the rat.

Three immunological responses normally present in the adult rat but absent or severely reduced in the neonatally thymectomized animal have been used to test the restorative ability of the thoracic duct cells. These immune responses are the capacity to undergo an Arthus and delayed hypersensitivity reaction under the proper stimulus and the ability to prevent development of tumor growth following inoculation with polyoma virus during the first week after birth. That the latter phenomenon does have an immunological basis seems to be reasonably well established(16). This is discussed later.

The present report describes attempts to restore neonatally thymectomized rats to immunological competence with thoracic duct