

METHOD OF CHRONIC ISOLATION OF SPINAL CORD SEGMENT IN THE ADULT CATS

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The current physiological literature reflects an increasing interest in isolated nervous structures. However, in the majority of papers, similar to reports from the 19th and early part of the 20th century, acute or semi-chronic preparations were investigated. A survey of the literature disclosed few works on chronically separated parts of the nervous system, and even less concerned with spinal cord preparations, particularly in adult cats (Goltz and Ewald 1896, Tower 1937, Tower et al. 1941, Clark 1940, Grayson and McCouch 1947, Kellogg et al. 1947, Shurrager and Dykman 1951, Mark and Gasteiger 1953, Nesmeyanova 1955, 1957, 1959; Bard and Macht 1958, Kozak et al. 1962, 1965; McPhereson 1966, Szentagothai 1967, de Groat et al. 1969).

The reflex activity of preparations including a few spinal cord segments is relatively regular in the late postoperative period (Afelt 1970). The maintenance of neurones in the chronically isolated anatomical segment has been described (Anderson 1963, Szentagothai 1958, 1964, 1967).

Therefore it was assumed that in this type of preparation many problems concerning not only the structure of the propriospinal segmental system but also its functions could be investigated.

The present report describes a surgical procedure for the isolation of the cat's spinal cord segment. In addition, a description is given of the methods of postoperative care allowing for long survival (more than a year) of the animal in reasonable health.

SUBJECTS

The description is based on 37 subjects. A good condition of the animals to be operated on appeared to be of critical importance for postoperative survival. Poor preoperative condition of some of our operated cats seemed to be a satisfactory explanation for mortality cases. Animals living for some months in the animal house, well adapted to the laboratory conditions and well fed (vitamins) showed much greater resistance than those after quarantine only.

As it has appeared from our experience, postoperative mortality may be considerably lowered if adult (1.5–3 years) completely healthy, castrated male cats whose weight varied from 4 to 5 kg were used for surgery (Fig. 1).

SURGERY

Operations were carried out under aseptic conditions and barbiturate general anesthesia (Nembutal 35–45 mg/kg). After removing the proper spinous processes two small holes were made in the adjoining vertebrae. The dura was opened and then the local anesthesia was administered: Novalgin (1–2%) was poured on the cord for 2 min. The spinal cord was twice transversely transected first at the upper level and then below. The dorsal and the dorsolateral tracts were cut at once with scissors. The gray matter was cut by the thin tip of the suction apparatus, and the remaining part of the white matter was transected gradually with jeweler's forceps. Since the operative area should be clearly seen the nervous tissue was cranially and caudally pushed away with blunt spatulas. Two–three dorsal rootlets were divided when they obscured the field of operation. The special spatulas, bent so as fit to the shape of the given spinal cord segment, were inserted between the roots and the cord on the intervention level, which protected the roots during the operation.

The anterior spinal artery and vessels of the anterior and posterior roots should be left intact. The posterior spinal artery was transected twice far from collaterals. Bleeding from the injured vessel was prevented by application of very small pieces of gelfoam. Usually after a few minutes after transection the blood circulation in the severed vessels of the isolated segment could be observed.

A ring of microfilter (Millipore Filter Corporation, Bedford, Mass.) (Basset and Campbell 1962) was inserted between the transected ends of the spinal cord. A sheat of filter was usually fitted over the exposed dura under the bone defect.

The wound was sutured using normal procedures and then antibiotics were administered. No heating of the animal was used during the operation.

Comment. It was noticed that (i) local anesthesia (ii) quick transection of the great ascending tracts and (iii) relatively low temperature of the operated animals during the operation successfully diminished hemorrhages. Care should be taken to avoid injury of the blood vessels. A chronically isolated segment may degenerate after concomitant alterations in the vascular supply. As it is known the dorsal branches of the spinal arteries follow the dorsal nerve roots to the spinal cord, where they are dissipated without a continuous dorsolateral trunk being formed. Consequently it may be assumed that described in this operation the transection of the "dorsal spinal artery" does not very strongly disturb the blood circulation of the isolated segment, provided only that the collaterals of this vessel were preserved.

The transected walls of the canalis centralis may close up, when the stumps are put together, and when the ring of filter separating the transected ends of the white matter is used. The diminution of the scar tissue (filter under the bone) augmented the chance of good circulation of the cerebrospinal fluid and then diminished the possibility of dilatation of canalis centralis. It was difficult however to evaluate the degree of the occlusion of the canalis centralis. The hydromyelia was observed in some preparations (Fig. 2).

The main attempt during the operation was careful sparing of the input (anterior roots) and output (posterior roots) from the isolated segment. In the first series of operations (11 cats) 50% of the transections at the levels in which the dorsal and ventral roots were long and spread over the surface, were incomplete (e.g. Fig. 3). Taking this into account, in the next series of operations, the spatula was inserted between the spinal cord and the roots, which enabled both the protection of roots and complete transection of the spinal cord.

Use of the ring of filter should result in an accurate postmortem identification of the transection level. We have had equally good results leaving the bone defect open, but in this conditions in the late postoperative period the difficulties in the roots preparations were observed.

POSTOPERATIVE CARE

From 30 to 60 min after operation the cat was kept warm (rectal temperature 38–39°C) and turned side to side several times a day to prevent development of hypostatic pneumonia. After 2–3 days the animal was moved from its hammock to an empty room. In order to

preserved the gradient of the environmental temperature, the heat source was suspended above the floor. This is very important on the first postoperative period. Later on, the optimal temperature for the spinal cats was 23–26°C.

In order to prevent dehydration, water or other fluids were served in the first day. In the second and third days 20–40 ml physiological salt solution with glucose were administered.

Feeding was started 2–3 days after the operation. The animals were given cooked and fresh ground horse meat, fresh fish, yolks, sour milk ad libitum (minimum 10 ml a day) and a live mouse from time to time if discorders in the digestive tract recurred. In the first days after the operation constipation occurred and them enemas and paraffin oil per os were administred.

During the whole postoperative period at least twice a day complete emptying of the bladder was induced by manual pressure. The bladders were extremely difficult to evacuate for the first three days. In this period 10–15 min before evacuation anesthetic and antispasmodic drugs (Novalgin 0.005 mg/kg) were given in our operated cats. Catheterization and irrigation with bladder antiseptics were applied if impactio urethrae was observed. It was noticed that in the late postoperative period this block seldom appeared in the castrated animals. In many of our operated cats, especially immediately after operation, hematuria was observed. It should be added that the hematuria itself may not necessarily be interpreted as an indicator of inflammatory processes. Vitamin K (0.002 mg/kg) and vitamin C were administred if hematuria was found. If after 24 hr of treatment improvement was not visible chlorpromasine (Fenactil maximum 0.001 mg/kg), Nitrofurantoin (0.003 mg/kg) or antibiotics were given. Relaxation of the vesical sphincter and annoying dribbling, pain evoked by manual pressure on the abdomen, erythrocytes in the urine and raised temperature indicated inflammation of the lower urinary tract. The treatment used in the human clinic was adopted. Usually weak herbal diuretics were admixed to the food.

A thick layer of sawdust on the floor of the room in which the animals were kept diminished the possibility of pressure sores. Local symptomatic treatment by protective and antibacterial drugs or anti-septics with ungentum cortisone drugs were administred if injuries of the skin appeared. In the late postoperative period alopecia localis was observed in some of our cats. Sometimes the area of these changes corresponded to segmental innervation.

As it is known the cat does not clean the anesthetized parts of its body. Therefore in order to keep the distal part of the body clean in

spinal cats, the technicians cleaned, washed, dried and brushed the cat if it was dirty. Castration facilitated cleaning of the perianal region.

Comment. Injuries of the periphery modify the reflex assembly evoked from the periphery (Magnus 1924). Repetitive stimulation of receptive fields of particular reflexes caused long-lasting changes in these reflexes (Sharpless 1964). As it is known from human clinic so called referred pain is evoked from impulses set up in the viscera and transferred in part to somatic pathways. All the above mentioned factors may alter the functional state of isolated nervous structure in our operated cats. Therefore it is important not only to obtain satisfactory postoperative recovery but also to prevent secondary postoperative changes such as skin injuries and pathological states in viscera. Generally the chronic spinal cats were treated like chronic patients with diseases in the urinary and digestive tracts and with a tendency to trophic skin changes. The use of sour milk in order to maintain intestinal flora and limitation of carbohydrates in the food were sufficient to bring about recovery and keep the alimentary tract in good condition. Taking care of the animal's skin and providing the cats with conditions in which the probability of skin injury is very low, could prevent serious trophic skin alterations. On the contrary there was no effective treatment for the lower urinary tract.

The effect of these treatments, and the condition of the experimental animals are summarized as follows:

1. Two small sickly cats died shortly after operation.
2. Two cases of renewal of tuberculosis were observed as was shown by postmortem examination.
3. Two cats were lost in which fine congenital abnormalities were observed (e.g. ankyloblepharon).
4. Six animals died as a result of cystitis in various periods after spinalization (10–471 days).
5. Eighteen animals were sacrificed: 1 after 3 months, 14 after 5–6 months, 2 after 8–9 months, 1 after 1 year.
6. Seven cats are alive more than 1.5 year.

ANATOMY

At autopsy the animals were anesthetized with Nembutal and the cord fixed *in situ* by perfusion of the whole animal. The spinal cords of dead animals were removed and fixed in 10% neutral formalin.

The processes of cicatrization of the injured sites were different in various preparations. After an opening in the vertebra 4–5 mm in length and after complete symmetrical transection, the scar growing into the

spinal cord was about 1.5–3 mm in length and covered the dorsal surface of the nervous tissue in the area corresponding to the opening (Fig. 4, cat 160). In the control experiment laminectomy was done and the isolated sector of the spinal cord was covered with muscular fascia. In this case, after 9 months, the segment appeared to be surrounded with connective tissue. Only 1/3 of the segment had approximately normal shape (Fig. 5, cat 541).

The dura mater usually coalesced with the scar tissue. Depending on the degree of isolation (filter sheets) the dorsal roots on the scar levels were more or less surrounded with connective tissue (Fig. 6A).

The isolation of one anatomical segment is possible after the opening in the two adjacent vertebrae in the L_{1–3} levels. At the levels of vertebrae L_{3–5} in these conditions the isolated segment consisted of 2–2.5 anatomical segments.

The isolated region of the cord was made the object of histological study (23 cases survived more than three months). From all the cords the segment was cut serially transversely in 10 μ sections prepared by Nissl, Klüver, Weil and some of them by Häqqvist methods. On the first examination the sections were of normal appearance (Fig. 7D). There was no visible decrease in dimension and no dissymmetry of the isolated segment. In the majority of cases 1.5 mm from the scar, the nervous tissue possessed an internal morphology comparable to that of the normal cord (Fig. 7CE). In some preparations 0.5 mm from the impairment the elements of this morphology could be observed (Fig. 7BF). In some cases cavities and other areas of destruction were found both at the level of operational opening and in the vicinity of the original trauma (Fig. 6bc). A great number of glial cells both in the gray and white matter were observed.

Comment. The lumbo-sacral part of the cat's spinal cord differ in individual animals (Romanes 1951). The same is observable in spinal cord-vertebra relations. Therefore the spinal cord segment isolated without visual control may be accurately identified only by postmortem examination.

The scar tissue formed partly by the bone and muscle elements which migrated to the region of the original trauma was mostly limited to the area under the bone defect. It should be stressed that a lack of the pronounced deformations of the isolated nervous tissue was observed at the levels in which the bone was preserved (Peele and Windle 1946). It is assumed that the pathological changes seen at some distance from the intended section are due to damage of the small blood vessels. Nevertheless, the traumatic degeneration extending from the performed lesion could not destroy the entire isolated sector.

The anatomical analysis of the effect of chronical isolation of spinal cord segment will be described elsewhere (Afelt, in preparation).

SUMMARY

Surgical procedure for the isolation of the adult cat spinal cord segment and the method of postoperative care allowing several months survival of the operated animals were described. The preliminary anatomical examination of the 23 preparations showed that the isolated structures survived and no pronounced morphological anomalies were present.

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Fig. 1. Cat no. 430 photographed 360 days after operation. On the diagram his spinal cord with two complete transections at L_2 and L_3 dorsal roots entrance level.

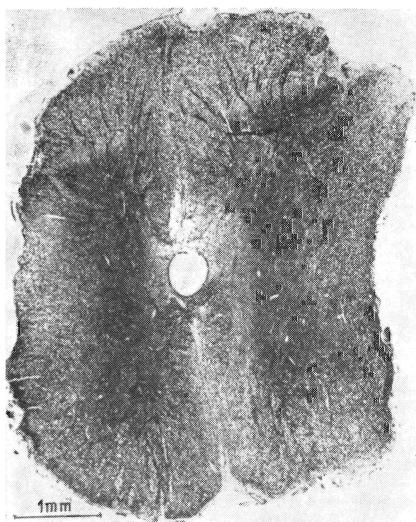


Fig. 2. Cat no. 156 sacrificed 176 days after operation. Low magnification of the Klüver-stained slide from isolated segment L_{5-6} . See dilatation of canalis centralis.

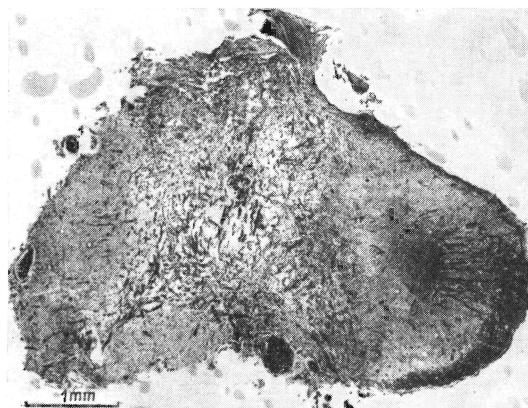


Fig. 3. Cat no. 547 sacrificed 167 days after operation. Microphotograph from Woelcke-stained slide at L_{6-7} transection level. The right ventrolateral part of the white matter was left undamaged.

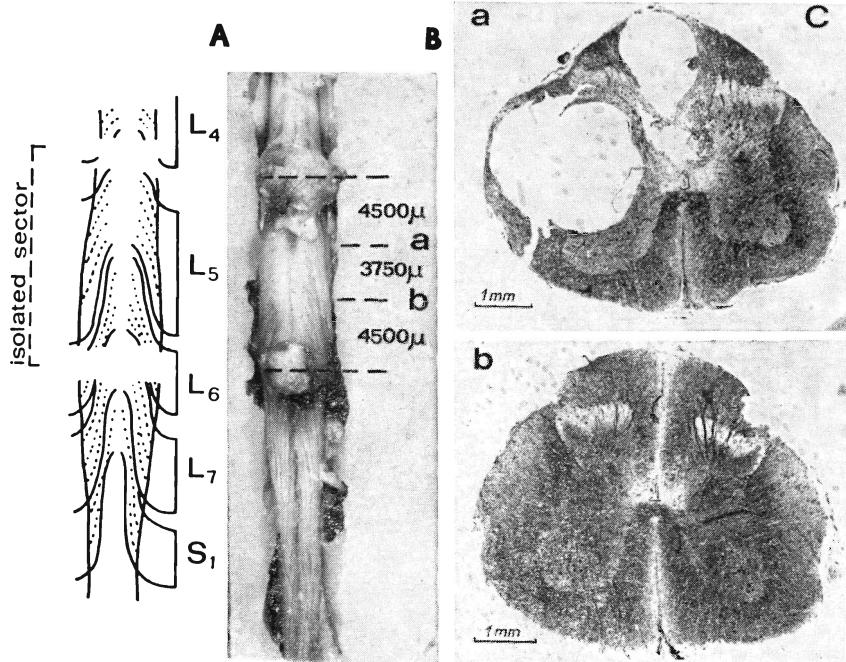


Fig. 4. Cat no. 160 sacrificed 155 days after operation. A: Diagram of the lumbo-sacral part of the spinal cord. Isolated sector marked by dashed line. B: Photograph of the same region. The scar tissue covered the two transection areas. a and b marks the level of cross sections seen in C and their distance from the scar. C: Cross sections of the isolated segment at the L_5 (a) and L_6 (b) levels (Klüver method). Note the cavities in the slide a.

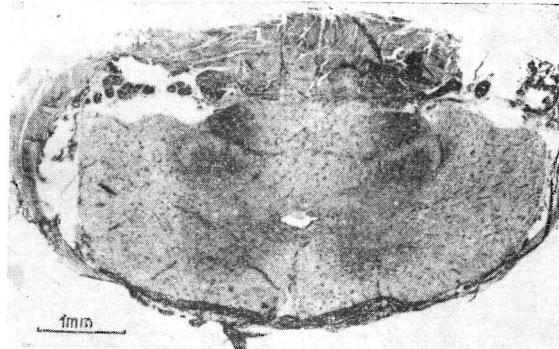


Fig. 5. Cat no. 440 sacrificed 269 days after operation. The control surgery with laminectomy (see text). The Klüver-stained slide from the least changed part of the isolated L_2 segment.



Fig. 6. Cat no. 160. The ventral L_4 roots on the transection level. At this level of transection the filter was not used.

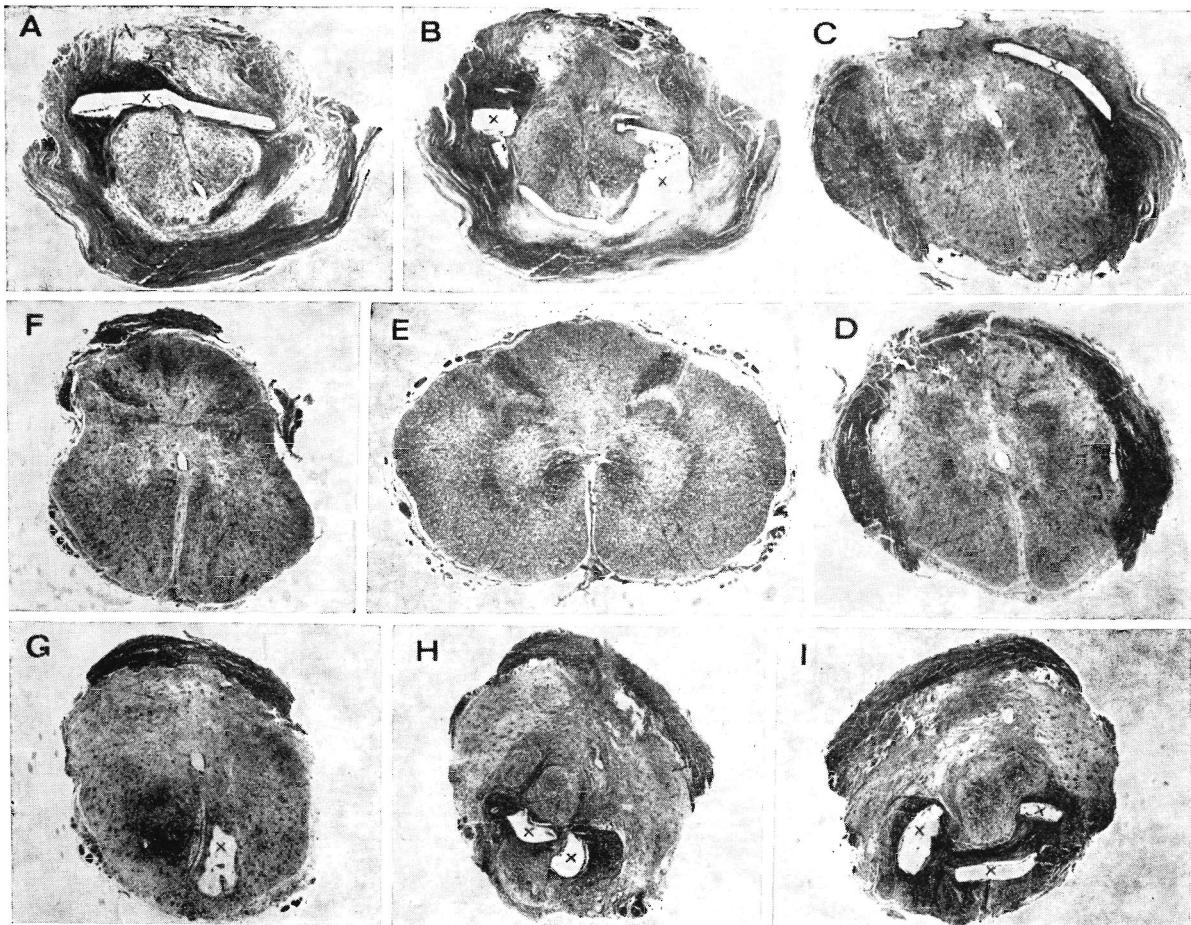


Fig. 7. Cat no. 180 died 120 days after operation (cystitis). A and B: Slides from the upper transection level. The filter sheets are marked by crosses. C: The cross section 500 μ below the B. D: The cross section 1400 μ below the B. E: The medial part of the isolated sector, this, is about 7 mm distance from the scars. F: The cross section 1500 μ from the H. G: The cross section 750 μ from the H. H and I: Slides from the lower transection level.