Brain and spinal cord lesions in the newborn rat

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Summary

Previous studies involving the induction of central nervous system lesions in animals have required complex, expensive equipment including stereotaxis and an operating microscope. This study describes a technique for producing telencephalic (forebrain) lesions in the developing rat by the use of copper wire. In addition, a nonvisual method of producing spinal cord transections in the young rat is discussed. Both techniques have a high success rate and minimal mortality.

Investigations on the developmental patterns of muscle morphology and histochemistry in newborn rats have shown progressive alterations for approximately 30 days after birth. By creating upper motor neuron lesions at various levels of the neuraxis, the influence of suprasegmental pathways on these alterations can be analyzed. The purpose of this report is to demonstrate a successful technique of producing brain lesions and spinal cord transections in rats 2-20 days old by instilling copper wires through the intact skull into the cerebrum, or by spinal cord transection by blind instrumentation through a skin incision. Pathological verification is demonstrated by gross and microscopic tissue analysis.

Materials and methods

Cerebral lesions

24 Sprague-Dawley infant rats (pups) were used. They were aged to within 12 h by inspection of the pregnant females twice a day. At the time of the procedure the age varied from 1 to 5 days. The pups were anaesthetized with intraperitoneal ketamine hydrochloride in concentrations varying from 1:25 to 1:40. Anaesthetic complications were kept at a minimum using a low quantity and strength (0.05 ml of 1:25). Subsequent studies of spinal cord transections indicated that methoxyflurane inhalation anaesthesia was safer than any quantity of ketamine hydrochloride.

For the 1st litter, a 0.56 mm diameter (24 gauge) coated copper wire was utilized after removal of the protective coat with sulphuric acid-potassium dichromate mixture. 0.46 and 0.31 mm diameter (26 and 30 gauge) non-coated copper wires were inserted in the 2nd and 3rd litters. Minimal sterile precautions involved soaking the wire and instruments in benzalkonium chloride, the use of sterile gloves, and skin preparation (no shaving was necessary).

The skin over the top of the skull was pulled taut so that the sutures became readily palpable. By this technique the bregma was readily identified (Fig. 1). A single short (approximately 2 cm) wire was inserted through the skin of the posterior frontal region, angled anteriorly and pushed by finger tip through the intact skull until encountering the resistance of the floor of the anterior fossa. The wire protruding from the surface was cut as short as possible, and a similar procedure was performed on the opposite side. After 2 h in the warm unit isolated from the mother, the pup was returned to the litter. Normal nursing patterns ensued a few hours later, and all but 4 of the pups survived and remained healthy until killed 4-25 days later.

The cerebral lesion was fixed in 10% formalin for 24 h. The skull could then be removed without fear of damage to the brain. Prior to sectioning, the brain was placed in ethanol overnight then passed through a series of alcohol, xylol and paraffin wax baths prior to embedding in paraffin wax for sectioning at 5mm thickness. The tissue was stained with Harris' haematoxylin and eosin, and luxol fast blue for myelin.

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Spinal cord transections
32 Sprague-Dawley rats from 5 litters were used. They were aged as described for the cerebral lesions. Only methoxyflurane inhalation anaesthesia was used.

The 2 lowest ribs were palpated. A line connecting these 2 points intersected with the vertebral column and this level was used as the site of entry into the spinal cord. A 2 cm vertical skin lesion was made over the midline, and the spinus processes were exposed by gentle, blunt dissection. Both vertebral arches were cut by shallow insertion of a scalpel blade (no. 11). The arches were severed one at a time while minimizing blood loss by frequent tamponade.

Next, a small, slightly sharpened spatula was inserted through the bony opening. It was advanced until firm resistance indicated the presence of the vertebral bodies. Then, by a slow back-and-forth pivoting motion, the spatula was moved through the spinal cord several times.

Fig. 2. Normal adult rat soleus muscle in cross section. Note the relative similarity in fibre diameters. Modified Gomori trichrome stain. × 300.

After the instrument was removed, the wound was compressed with sterile gauze and 2 silk sutures (5-0) closed the incision. The animal was placed in a warm incubator for 2 h to recover from the anaesthetic, then returned to the litter with the lactating mother.

Soleus muscle histology was examined utilizing the modified Gomori trichrome stain (Engel & Cunningham, 1963) to demonstrate the morphology of the muscle fibres (Fig. 2). By this technique an animal from the 1st litter showed grouped fibre atrophy indicative of lower motor neuron dysfunction (Fig. 3). Accordingly, dissections were undertaken in a 14 and a 20 day old rat to correlate topographical landmarks with spinal cord levels. Prior to dissection, the vertebral bodies identified by the intersection of a line drawn between the 2 inferior ribs were marked. The lumbosacral enlargement was intersected by this vertebral level (Fig. 4). Following this study, all subsequent spinal cord transections were carried out 1-2 cm above the intersection line. Animals under 7-10 days of age required the shorter distance.

Results
Cerebral lesions
Of the 24 animals 4 died and 13 were killed before they were a month old. The remaining 7 were observed for at least 30 days for local or systemic

Fig. 3. Soleus muscle of a rat with a cord transection through the lumbosacral plexus. Note the fibre size variation and a group of small fibres suggesting neurogenic atrophy. Modified Gomori trichrome stain. × 300.

Fig. 4. Dissection of vertebral arches to demonstrate posterior view of the spinal cord. A cord transection at T5-6, B inferior rib intersection line at L1, C lumbosacral enlargement from vertebral levels T11 to L3.
side effects of the operative procedure. The cause of death of the 4 pups could not be determined. The other 20 animals remained healthy throughout the observation period. Most had locomotion difficulty during the 1st post-operative week, but the disability lessened as time passed.

The brains of the sacrificed animals were fixed and examined by gross and microscopic inspection. Thick sections of these brains showed large, cyst-like lesions surrounding the copper wire (Fig. 5). There was no evidence of local infection and the underlying cranial vault was intact. The animals with the longest post-operative period had the largest brain lesions. However, the size of the lesions was not always consistent. In one animal a 15 mm lesion was found on the left with 75% of the hemisphere destroyed on the right.

The coronal sections shown in Fig. 5 reveal large areas of destruction involving the cerebral cortex, posterior thalamus and hippocampus. Each of these specimens had bilateral wire instillation and the adjacent tissue is undergoing necrosis as indicated by cyst formation with reactive gliosis and white-cell infiltration.

**Spinal cord lesions**

Of the 32 animals studied, 7 died during surgery or within 6 h. 3 of these were anaesthetic deaths and the remainder were due to surgical trauma. Of the 25 survivors, 9 had some degree of permanent paralysis. An additional animal was paralyzed for only 24 h and then recovered completely from a post-operative paraparesis. 4 had predominantly a monoparesis and 5 a paraplegia. Some had incomplete cord transections, indicated by varying degrees of hind leg weakness. All paralyzed animals were readily accepted by the mother for feeding; however, the growth of some was less than litter mates, suggesting inadequate nutrition.

Fig. 4 demonstrates the topographical anatomy of the spinal cord of a 20 day old rat. A line drawn between the 2 inferior ribs intersects with vertebral level L1. The conus medularis (caudal region of the cord) is found at L6, and the lumbosacral enlargement between T11 and L3. The cord transection lesion is shown at vertebral level T5-6.

Histological confirmation of cord injury is demonstrated in Fig. 6. Sections above the lesion showed evidence of chromatolysis indicating injury to intercostal nerves adjacent to the transection. Haemorrhage and, finally, almost total cord destruction was found in the area of maximum injury.

**Discussion**

Previous studies involving the introduction of brain and spinal cord lesions in experimental animals
have utilized relatively complicated techniques (Buller, Eccles & Eccles, 1960; Konig, 1963; Massopust, 1956; Stelzner, Ershler & Weber, 1975). The techniques described here offer relatively simple methods of producing major brain and spinal cord lesions in the developing rat. For best results, the surgery must be performed rapidly to decrease the incidence of anaesthetic or surgical death. Once beyond the early post-operative period (24 h) the survival rate of both techniques was 100%.

Previous reports documented methods of producing brain lesions in the developing animal before birth. Dekaban (1969) produced major brain defects in the mouse foetus by accurate timing and dosage of irradiation during pregnancy. More than 80% of litter mates had brain lesions and the distribution of the lesions varied widely, with hydrocephalus the most frequent abnormality. This method induces total body irradiation so that all tissues are subjected to the side effects of the agent. A study of developmental patterns of the neuromuscular unit using this model would be of questionable value due to the direct effect of the irradiation on the lower motor neuron.

Warkany & Petering (1972) produced brain malformations in rat foetuses using a low-zinc diet during pregnancy. 70% of the 193 foetuses examined had gross or microscopic evidence of a brain abnormality. As with irradiation, all body tissues are exposed to this environment so that any interference with developmental patterns of the neuromuscular unit may be secondary to the systemic metabolic effect rather than to the brain abnormality.

Fischer, Sayer & Bickford (1957) pointed out the extensive reaction of cat brain to bare silver and copper wire. They demonstrated that within a week an intensive local inflammatory process was well under way. This area became walled off with fibroblasts by the 2nd week and a large area of central necrosis was found at 4 weeks. The mechanism of action may be electrolysis or reaction to a toxic metal salt. In the present study, bare copper wire was inserted in order to induce brain lesions without interfering with the development of the remainder of the central nervous system or the neuromuscular unit.

The spinal cord transection technique described here adds some useful topographical points regarding the anatomy of the rat spinal cord in relation to non-neural structures. Farris & Griffith (1957) indicate that the termination of the cord (conus medullaris) is found at vertebral level L4. Our study of rats aged 14 and 20 days places the conus medullaris at L6 and the lumbosacral enlargement between T-11 and L3. Since the lumbosacral outflow supplies all of the lower extremity musculature, it is critical to approach the cord above this structure in order to produce a purely upper motor neuron lesion.

Some of our earlier animals had strong evidence for lower motor neuron abnormalities indicated by atrophic muscle fibres (Figs 2 and 3). Subsequent dissections (Fig. 4) pointed out that a line drawn between the 2 lowest ribs intersects vertebral level L1. A lesion placed here results in a combined upper and lower motor neuron deficit because of injury to the lumbosacral outflow to the lower extremity. Accordingly, in a 2-3 week old rat a point approximately 15-20 mm above the intercept line results in a mid-thoracic lesion. For animals
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less than a week of age, 10-15 mm above the intercept line is adequate.

The frequency of successful surgery is quite different for the 2 techniques described. 80% of the animals with copper wire instillation suffered major brain insults. However, only 9 out of 25 animals who survived cord transection surgery had paralysis with corresponding cord lesions. The difficulty with this 'blind' procedure lies in the careful balance between too vigorous an incision, with a resultant intraabdominal hemorrhage, and a technique which is too shallow to produce the lesions. As experience was gained the success rate improved, but 50% effectiveness was the highest rate possible.

In summary, 2 techniques for the induction of central nervous system lesions in the rat have been presented. By these methods, it is possible to obtain major defects in the telencephalon and spinal cord with a high percentage of success and a minimum of mortality. No sophisticated equipment is required and the operated animals provide good models for studying post-injury developmental patterns in the neuromuscular unit.

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References


