Dev. Neurosci. 2: 115–121 (1979)

Development of Rat Spinal Cord

I. Weight and Length with a Method for Rapid Removal[1]

Byron N. de Sousa and Lloyd A. Horrocks

Department of Physiological Chemistry, Ohio State University, Columbus, Ohio

Key Words. Spinal cord · Maturation · Development · Removal · Weight · Length

Abstract. At birth, the rat spinal cord has 5% of the adult weight and 21% of the adult length. The ratio of weight to length, the 'thickness', more than doubled between 7 and 20 days of age and doubled again between 20 days and adulthood. The intact spinal cord can be removed from rats of any age within 1 min. After decapitation and partial dissection of the vertebral column at the sacral level, the spinal cord is ejected by means of hydraulic pressure from a syringe. Use of this method avoids prolonged dissection of the vertebral column with associated anoxia and trauma.

Introduction

Donaldson [7] reviewed the maturation of the spinal cord of the laboratory rat many years ago. Since then, improved nutrition has increased the growth rate of the rat. Because the size of the spinal cord is related to the size of the carcass, a new study of spinal cord development was necessary. The mammalian spinal cord has been a difficult tissue to study because it is enclosed within a large number of vertebrae and it is extremely sensitive to trauma. Dissection of the spinal cord from the vertebral column requires at least 20 min for the adult rat. This dissection is inevitably accompanied by multiple traumatic injuries to the cord plus the insult of ischemia for a 20-min period. Ischemia can be avoided by maintaining the animal with a respirator during removal of spinal cord tissue. This method is time-consuming, difficult, and introduces the variable of anesthetic agents. Another possibility is freezing of the whole animal or vertebral column in liquid nitrogen, but freezing of the spinal cord is not immediate and removal of the frozen spinal cord is quite difficult. In this paper we describe a method for removal of the intact spinal cord in less than 1 min with minimal trauma. The method

[1] This study was supported in part by Grants NS-10165 and NS-08291 from the National Institute of Neurological and Communicative Diseases and Stroke, US Public Health Service.

was used to obtain measurements of weight and length of rat spinal cords during maturation.

Materials and Methods

Sprague-Dawley (Madison, Wisc.) rats were decapitated at 0, 1, 2, 3, 5, 7, 9, 11, 13, 14, 15, 17, 20, 21, 25, 30, 40, 58, 90, 127, and 190 days of age. Two parallel longitudinal incisions were made on each side of the vertebral column at the sacral level. After insertion of forceps to hold the vertebral column, it was transected at the most distal position. For older animals, longer incisions along the vertebral column are necessary in order to sever more of the spinal roots. Selection of a syringe and needle depends on the age of the rat. For rats older than 50 days, a 20 cm^3 syringe is used. Syringes of 5 or 10 cm^3 capacity are used with smaller rats. The needle should approximate the inside diameter of the vertebral canal. Up to 7 days of age, we use 20-gauge needles, then 18- or 19-gauge needles for rats of 7–21 days of age. For adult rats a 16-gauge needle is necessary. For younger rats we have also been able to use plastic tips for micropipettes after removing a portion of the large end so that the remainder fits inside a Luer-Lok hub.

A gauze pad is placed immediately in front of the rat. The syringe is filled with cold water, the vertebral column is held with forceps and the syringe needle is introduced about 5 mm into the caudal end of the vertebral canal. Pressure is then applied to the syringe plunger with the thumb until the spinal cord is ejected from the vertebral canal (fig. 1). The cord is blotted on the gauze and immediately placed in a Petri dish. Graph paper under the dish was used to measure the length of the cord. The adhering meninges were dissected off, any remaining spinal nerves were trimmed, then the spinal cord was placed in a freezer (–20 °C) until required for chemical determinations. Weights of the tissues were determined after freezing. With appropriate modifications of the syringe needle, this method may also be used with mice and cats.

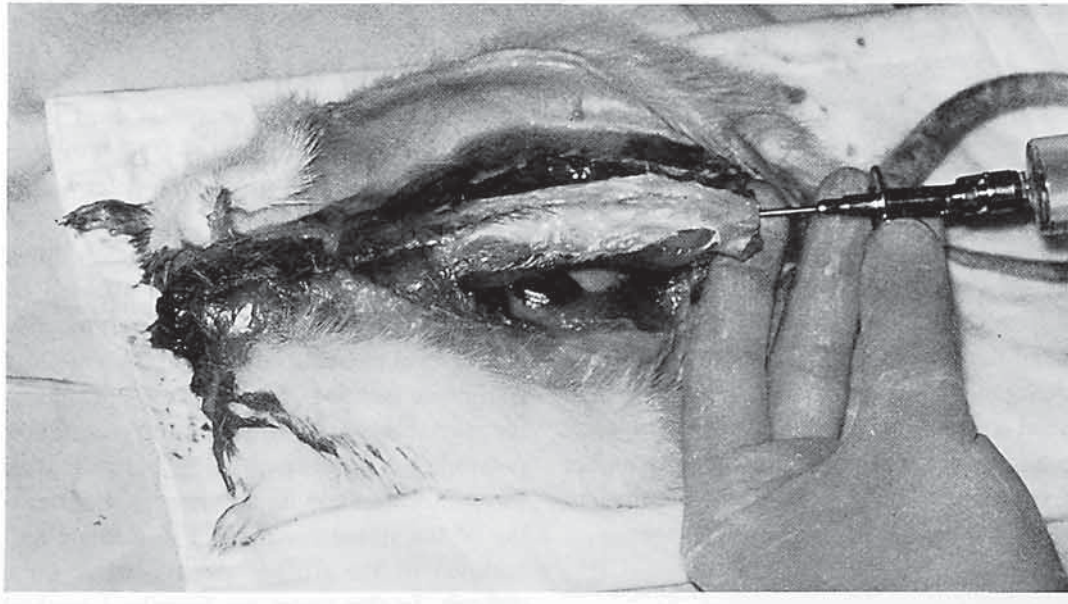

Fig. 1. The carcass of an adult rat just after the ejection of the spinal cord by hydraulic pressure from a syringe.

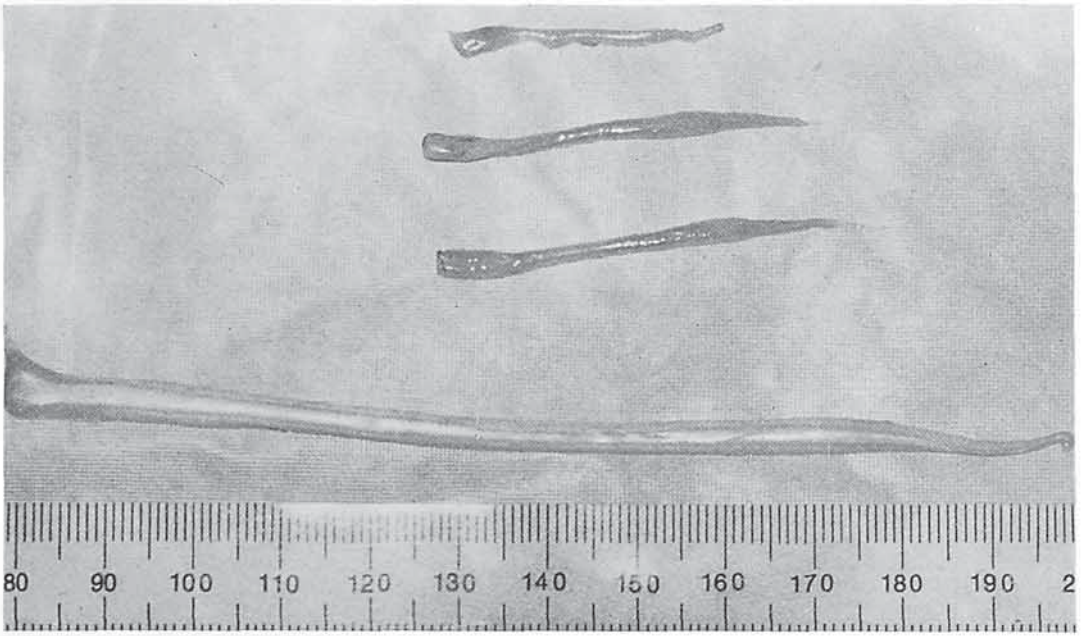

Fig. 2. Ejected spinal cords. From top to bottom, the rats were 3, 7, and 7 days of age and adult.

Results

Intact spinal cords were ejected by hydraulic pressure (fig. 2). The ejected material included the meninges and portions of many of the spinal roots. An occasional spinal root was torn from the cord with loss of a small amount of tissue from the dorsal horn. The microscopic appearance of the spinal cord was normal (fig. 3).

The rat spinal cord increased from 34 mg and 25 mm at birth to 743 mg and 120 mm in the adult (127 days of age). The weight of the spinal cord at birth is only 5% of the adult weight (fig. 4). A marked increase in weight began during the third week of life. From 14 to 21 days, the weight nearly doubled. The spinal cord length at birth is 21% of the adult length (fig. 5). A gradual increase during development was observed. Since changes were taking place in both the weight and length, we also plotted the ratio of weight to length as a measure of 'thickness' of the spinal cord (fig. 6). This parameter more than doubled between 7 and 20 days of age, then doubled again between 20 days and adulthood. Between 11 and 120 days of age, the increase of 'thickness' was proportional to the logarithm of the age. Part of this increase is due to the deposition of myelin [4]. Partly because of the pronounced change in length of the spinal cord during development, the change in weight is much greater for the spinal cord than for the brain. However, even the 'thickness' parameter, mg/cm of spinal cord, increased several-fold during the course of development. Increases in the 'thickness' began shortly after birth, were of greatest magnitude during the third week of life, then gradually diminished but with detectable increases beyond 100 days of age.

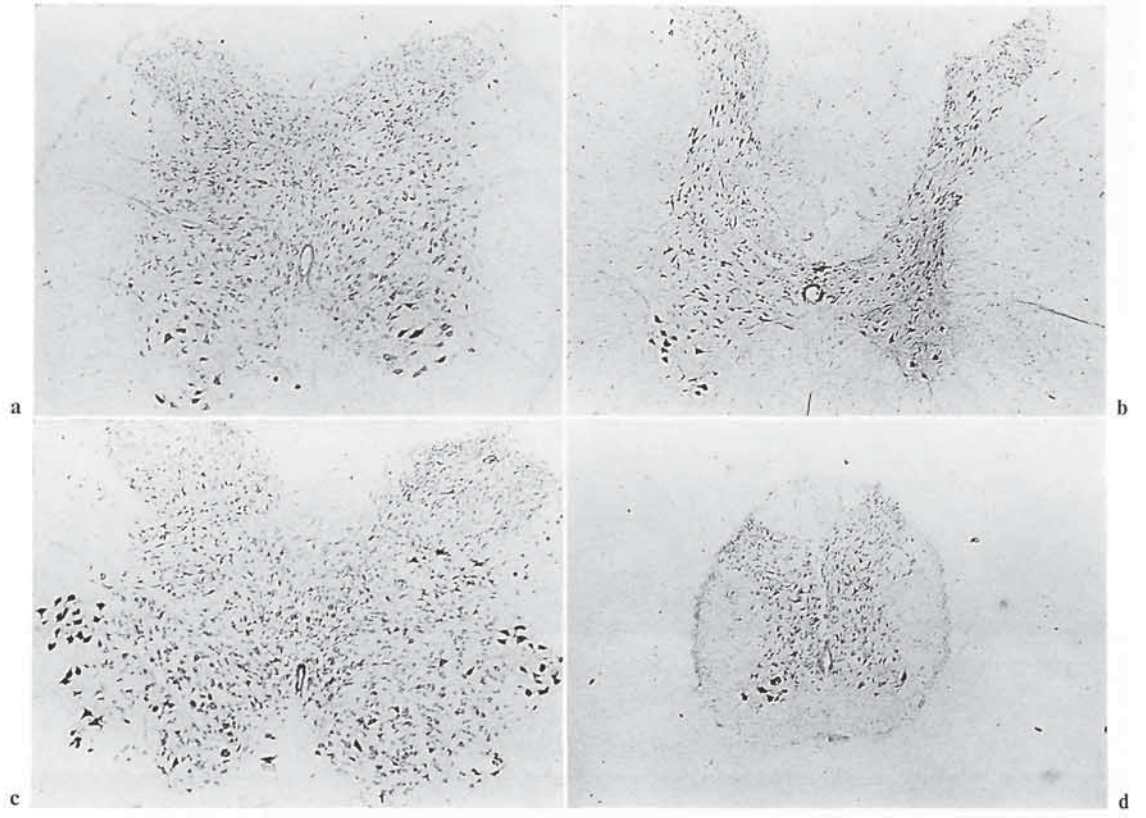

Fig. 3. Transverse sections of ejected spinal cords. Representative sections were cut from cervical (a) thoracic (b), lumbar (c) and sacral (d) levels of the spinal cord. The entire spinal cord was immersed in 10% buffered formalin for 1 week following the rapid removal described in this account. Representative blocks (1 cm in length) from each cord level were embedded in paraplast following routine graded ethanol dehydration. Transverse sections were cut at 12 μm and stained with cresyl violet. ×130.

Discussion

Removal of the spinal cord by pressurized ejection is much faster than previous methods which require dissection of the spinal cord from the vertebral column. By analogy with brain, we presume that energy supplies are depleted [12], free fatty acids are released [2, 3], mitochondria are uncoupled [15], and many other metabolic changes take place in the tissue during the time required for dissection. The present method may be used to study the effects of stagnant anoxia on spinal cord [17], and is recommended for biochemical and other studies in which perfusion fixation is not possible.

Spinal cord tissue has been removed from chick embryos by compression of the neural

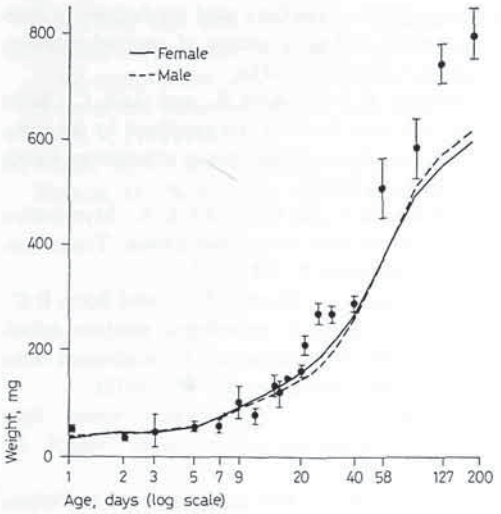

Fig. 4. The weight ± SEM of rat spinal cords during maturation. Note that the age scale is logarithmic. The continuous lines represent the weights in table 157 in Donaldson's review [7]. The number of spinal cords varied from 5 to 17 from birth to 20 days of age and from 3 to 7 from 21 to 190 days of age.

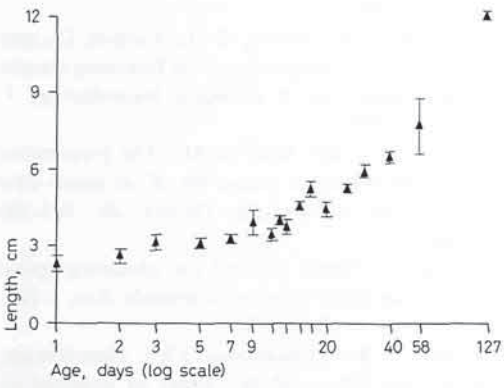

Fig. 5. The length ± SEM of rat spinal cords during maturation.

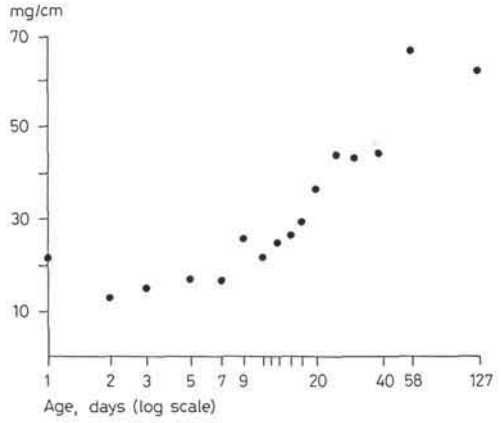

Fig. 6. The 'thickness' (ratio of weight to length) of rat spinal cords during maturation.

canal with 2 pairs of forceps moved alternately in the direction of the sectioned vertebral column [13, 16]. This 'toothpaste tube' method compresses part of the cord and is suitable only for young animals. *Hudson and Kini* [10] removed the vertebral column and cut it transversely into four parts. The spinal cord was removed by pushing a tightly fitting probe through the vertebral canal. *Levine* [11] described a method for removal of the vertebral

column from the spinal cord after fixation. For nonhistological purposes he recommends blowing the cord out of the spinal cord with a blast of air, but no description or results were provided. In our hands, hydraulic pressure from a syringe is much more reliable than pneumatic pressure.

The spinal cord is extremely sensitive to impact trauma and compression [6, 14]. Among the sequelae are hemorrhagic necrosis of the gray matter [8, 18], demyelination of the white matter [5, 9] and a permanent loss of function in areas below the site of injury [1]. Compression and trauma of the cord are unavoidable during removal by previous methods. With removal of the spinal cord by pressurized ejection, any injury to the cord occurs immediately before the intact cord is obtained and the cord can be frozen or otherwise processed within seconds. Thus the ejection method minimizes pathologic changes in the tissue and is simpler and faster than any other method.

Growth of the spinal cord during the suckling period was nearly identical to that reported by *Donaldson* [7]. After weaning, the weight of the spinal cord increased faster in our rats. In the adult rat (127 days of age) the weight of the spinal cord was 33% larger in the present series as compared with Donaldson's results. The greater mass of the spinal cord in the present animals is very likely a result of improved nutrition and the 133% larger body weight.

References

1 Albin, M.S.; White, R.J.; Acosta-Rua, G., and Yashon, D.: Study of functional recovery produced by delayed localized cooling after spinal cord injury in primates. J. Neurosurg. *29:* 113–120 (1968).

2 Bazan, N.G.: Free fatty acid production in cerebral white and gray matter of squirrel monkey. Lipids *6:* 211–212 (1971).

3 Cenedella, R.J.; Paoletti, R., and Galli, C.: Brain free fatty acid levels in rats sacrificed by decapitation versus focused microwave irradiation. Lipids *10:* 290–293 (1975).

4 De Sousa, B.N. and Horrocks, L.A.: Myelination of rat spinal cord during maturation. Trans. Am. Soc. Neurochem. *8:* 152 (1977).

5 Dohrmann, G.J.; Wagner, F.C., and Bucy, P.C.: Transitory traumatic paraplegia: electron microscopy of the early alterations in myelinated nerve fibers. J. Neurosurg. *36:* 407–415 (1972).

6 Dohrmann, G.J.: Experimental spinal cord trauma, a historical review. Archs Neurol. *27:* 1–29 (1973).

7 Donaldson, H.H.: The rat, Memoirs of the Wistar Institute of Anatomy and Biology; 2nd ed., vol. 6 (Sauders Philadelphia 1924).

8 Ducker, T.B. and Perot, P.L., Jr.: Spinal cord oxygen and blood flow in trauma. Surg. Forum *22:* 229–231 (1971).

9 Horrocks, L.A.; Toews, A.D.; Yashon, D., and Locke, G.E.: Changes in myelin following trauma of the spinal cord in monkeys. Neurobiology *3:* 256–263 (1973).

10 Hudson, A.J. and Kini, M.M.: The preparation and some metabolic properties of rat spinal cord slices. Can. J. Biochem. Physiol. *38:* 965–968 (1960).

11 Levine, S.: Rapid method for obtaining spinal cords from small laboratory animals. Am. J. clin. Path. *43:* 272–273 (1965).

12 Lowry, O.H.; Passonneau, J.V.; Hasselberger, R.X., and Schulz, D.W.: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J. biol. Chem. *239:* 18–30 (1964).

13 Maletta, G.J.; Vernadakis, A., and Timiras, P.S.: Pre- and post-natal development of the spinal cord: increased acetylcholinesterase activity. Proc. Soc. exp. Biol. Med. *121:* 1210–1211 (1966).

14 Osterholm, J.L.: The pathophysiological response to spinal cord injury. J. Neurosurg. *40:* 5–33 (1974).

15 Ozawa, K.; Seta, K.; Araki, H., and Handa, H.: The effect of ischemia on mitochondrial metabolism. J. Biochem. *61:* 512–514 (1967).

16 Pomerat, C.M.; Drager, G.A., and Painter, J.T.: Effect of some barbiturates on tissues *in vitro*.

Proc. Soc. exp. Biol. Med. *63:* 322–326 (1946).
17 Torello, L.A.; Yates, A.J., and Horrocks, L.A.: Lipid composition of rat spinal cords undergoing postmortem autolysis. Trans. Am. Soc. Neurochem. *9:* 111 (1978).
18 White, R.J.; Albin, M.S.; Harris, L.S., and Yashon, D.: Spinal cord injury: sequential morphology and hypothermic stabilization. Surg. Forum *20:* 432–433 (1969).

Dr. Lloyd A. Horrocks, Ohio State University,
Department of Physiological Chemistry,
1645 Neil Avenue, Columbus, Ohio 43210 (USA)