Postnatal Development of Tail Motoneurons and Muscles in Cat

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ABSTRACT. The postnatal development of motoneurons in two tail muscles, the extensor caudae medialis (ECM) and extensor caudae lateralis (ECL), and that of ECL muscle fibers were studied in cats 1 week to 2 months old. The location of ECL and ECM motoneurons in cats of different ages did not change postnatally. Somal size (average of maximal and minimal diameters) of ECL and ECM motoneurons gradually increased, and the size distribution of ECL was bimodal at 2 months. The activities of succinate dehydrogenase (SDH) and phosphofructokinase (PFK) were studied to determine oxidative and glycolytic activities in ECL growth. SDH activity rapidly decreased between 1 week and 2 weeks of age, while PFK activities increased gradually.—**KEY WORDS:** feline, motoneuron, muscle fiber, spinal cord, tail.

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The tail is a conspicuous part of cat's body. Adult cats show various tail movements to maintain body balance, signal mode to its congeners and so on [6, 9, 10]. However, immature cats can not freely perform finely controlled tail movements. After birth, tail movements develop as the cat grows. It could be considered that development of tail movements is accomplished by developments both of the neuronal system and the mechanical system (muscle, tendon and bone). Although some investigators showed the postnatal developments of motoneurons and muscle fibers, they concentrated on limb muscle and motoneurons [1, 7, 11–13, 15]. Few investigators have studied tail muscle and motoneurons. In the present experiment, we studied the development of tail motoneuron and muscle as cats mature.

MATERIALS AND METHODS

Thirty-eight cats with straight long tails were used for these experiments. The day of birth was designated as postnatal day 1. The motoneurons innervating the M. extensor caudae medialis (ECM) and extensor caudae lateralis (ECL), and the muscle fibers of ECL were studied in 5–9 cats each at 1 week, 2 weeks, 3 weeks, 4 weeks and 2 months of age.

The motoneurons were labeled using horseradish peroxidase (HRP, Sigma, U.S.A.) as retrograde tracer. A 30–35% HRP solution was directly injected into the muscle (6–25 μ l per muscle with multiple penetration) with a 25 μ l Hamilton syringe. HRP was injected into the ECM and ECL between the 1st (Ca 1) and 3rd caudal (Ca 3) vertebrate levels under anesthesia with pentobarbital-Na solution (35–45 mg/kg, ip). To prevent HRP solution leakage into other muscles, the muscle injected with HRP solution was separated from surrounding muscle without injuring the fasciae of the muscles before injecting HRP solution into the middle of the muscle, and then applied a layer of thin vinyl film over the injected muscle. Table 1

Table 1. Injection patterns of HRP solution

Injected muscles	1 wk	2 wks	3 wks	4 wks	2 months
IECM	1	0	0	0	0
IECL	1	0	0	0	0
IECL+rECM	3	2	2	2	2
IECL+IECM	2	2	2	2	2
IECM+rECM	2	2	1	2	2

l: left, r: right, wk: week, wks: weeks, number: number of cats.

shows injection patterns of HRP solution in 38 cats. After a survival time of 24 hr, the animals were deeply anesthetized with pentobarbital-Na and the animals were perfused transcardially with physiological saline (200–2,000 ml, 38°C), a fixative solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The spinal cord between S1 and Ca 4 were removed and their 60 μ m serial sections were cut in a transverse using a vibratome (Dosake, EM. Co., Ltd., Japan). HRP preparations were treated with tetramethylbenzidine (TMB) following the method described by Mesulam [8]. All sections were mounted on gelatin-coated glass slides and lightly counterstained with neutral red. The maximum and minimum diameters of labeled motoneurons with visible nuclei were calculated from areas of motoneurons using an image processing system (Toyo physical-25–512, Japan) connected with a personal computer (NEC, PC-9801, Japan) and averaged diameters of maximum and minimum diameters were shown.

The activities of two enzymes in ECL at the Ca 2–3 level, phosphofructokinase (PFK) and succinate dehydrogenase (SDH) were used to determine glycolytic and oxidative potentials, respectively. The tissue used for PFK activity was homogenized in ice-cold homogenization medium containing 150 mM KCl, 50 mM KHCO₃, and 6 mM EDTA (1:20 wt/vol). Tissue samples used to determine SDH activity were homogenized in ice-cold 33.3 mM

phosphate buffer (1:20 wt/vol, pH 7.4). PFK activity was assayed spectrophotometrically based on the rate of endogenous NADH oxidation. The FPK reaction medium consisted of 50 mM triethanolamine (pH 7.6), 5 mM EDTA, 10 mM MgCl_2 , 2.8 mM ATP, 2.8 mM fructose 6-phosphate, 0.3 mM NADH, 1 U aldolase, and a 17.1-U glycerol 3-phosphate dehydrogenase and 18.3-U triosephosphate isomerase mixture. Reaction was started by adding 20 μl of homogenate and followed for 3 min at a wave-length of 340 nm. SDH activity was determined using a spectrophotometric method described by Cooperstein *et al.* [5] with cytochrome *c* as the terminal hydrogen acceptor. SDH was assayed using 20 μl of tissue homogenate. The reaction medium for SDH consisted of 40.5 mM phosphate buffer (pH 7.4), 16.6 mM sodium succinate, 1 mM NaCN, 16.6 μM cytochrome *c*, 0.4 mM AlCl_3 and 0.4 mM CaCl_2 . The reduction of cytochrome *c* was followed for 3 min at a wave-length of 550 nm. All enzyme assays were performed in triplicate at 25°C, and the results were expressed in micromoles per gram of noncollagenous protein (NCP) per minute. A one milliliter of homogenate was used to determine NCP content.

Student's *t*-test was used to evaluate differences in the mean values.

RESULTS

Development of motoneuron: The locations of ECL and ECM motoneurons in cats of different ages are shown in Fig. 1. Each dot indicates a motoneuron. At all ages,

ECM and ECL motoneurons were located in dorsomedial and ventral areas in the ventral horn, respectively. It is evident that the relative position in the lamina IX of the ECL and ECM motoneurons did not change postnatally.

Figure 2 shows ECL and ECM motoneurons at 1 week (1 wk), 3 weeks (3 wks), and 2 months. In Fig. 3, dendritic distribution from ECL and ECM motoneurons at 2 weeks (2 wks) and 4 weeks (4 wks) are shown. At 1 wk, the neurons appeared as rounded, ovoid and triangular cells. Development of dendrites was not significant. Dendrites from ECL and ECM motoneurons were developed as the cat grew. Between 2 and 3 wks, significant development of dendrites was observed and dendrites from ECL motoneurons spread in dorsal and lateral directions, while dendrites from the ECM spread in all directions (Figs. 2 and 3). From 2 wks, dendrites from ECM motoneurons were observed crossing to the contralateral side. At 2 wks, dendritic connections between ECL and ECM motoneurons, or ECM motoneurons on right and left sides could not be observed (Fig. 3). From 3 wks, developments of dendritic connections were observed and numerous dendritic connections were observed at 4 weeks of age (Fig. 3a, b). During development, the shape of motoneurons changed from round or oval to polygonal with development of the dendrites.

Figure 4 shows changes in the sizes of ECL and ECM motoneurons. At 1 wk and 2 wks, histograms of motoneuron size of ECL and FCL motoneurons were unimodal. The average diameter of ECL and ECM motoneurons as well as the variability of diameter

Fig. 1. Location of ECM and ECL motoneurons on transverse section at Ca2 spinal segment obtained from 1 week to 2 months of age. Black dots indicate location of motoneuron cell bodies. Relative position in the lamina IX of the ECL and ECM motoneurons did not change postnatally. wk; week, wks; weeks.

Fig. 2. ECL (a) and ECM (b) motoneurons at 1 week, 3 weeks and 2 months of age (Ca 1). Development of motoneuron size and dendritic projection were observed. Scale: 40 μ m.

gradually increased. At 2 months, bimodal distribution was observed in the ECL motoneurons, however bimodal distribution of ECM motoneuron size was not observed. There was no significant difference between the size of ECL and ECM motoneurons.

Development of muscles fibers: Table 2 shows SDH and PFK activities in the ECL together with the ratio of SDH to PFK (SDH/PFK). PFK activity was significantly higher than that of SDH in all age groups ($p < 0.01$). In PFK activity, a tendency forward increased PFK activity with

age was observed, however the increases of PFK activity were not significant. SDH activity was markedly decreased between 1 wk and 2 wks of age ($p < 0.01$), but remained almost constant from 2 wks to 2 months. The ratio of PFK activity to SDH activity increased with age, especially between 1 wk and 2 wks ($p < 0.01$).

DISCUSSION

In the present experiment, HRP solution was injected

2 wks

4 wks

Fig. 3. The dendritic connection between ECM motoneurons on the left and right side, and ECM and ECL motoneurons on same side at 2 weeks and at 4 weeks of age. a and b in 4 weeks show dendritic connections between ECM and ECL motoneurons, and ECM motoneurons in left and right sides, respectively. cc: central canal, vf; ventral median fissure. Scale: 80 μ m.

into muscles to investigate the distribution of motoneurons. Another approach, applying HRP solution directly to cut endings of muscle nerves may be considered. Since ECL and ECM are innervated by numerous fine nerves [14], it is very difficult to apply HRP solution to muscle nerves. One potential problem of injecting HRP solution into the muscles is leakage of HRP solution to another muscle. In the present experiments we injected a highly concentrated HRP solution (30–35%) into the center of each muscle and covered the injected muscles

Fig. 4. Size (average of maximal and minimal diameters) distribution of ECL (right) and ECM (left) motoneurons grouped in bins of 5 μ m. The distribution broadened and shifted to the right with age. A bimodal distribution of ECL motoneurons was clearly observed at 2 months.

Table 2. PFK and SDH activities in ECL

age	PFK activity	SDH activity	PFK/SDH
1 wk (n=5)	149.9 $\pm$ 22.6	17.0 $\pm$ 3.2	9.1 $\pm$ 2.4
2 wks (n=5)	143.6 $\pm$ 21.0	8.2 $\pm$ 2.2	17.6 $\pm$ 2.2
3 wks (n=5)	151.3 $\pm$ 46.6	8.5 $\pm$ 2.7	17.8 $\pm$ 0.5
4 wks (n=5)	167.6 $\pm$ 22.3	9.2 $\pm$ 2.5	17.9 $\pm$ 3.5
2 months (n=6)	189.5 $\pm$ 45.8	8.7 $\pm$ 1.8	22.3 $\pm$ 8.2

Values are mean $\pm$ SE in μ mol·g noncollagenous protein $^{-1}$ ·min. $^{-1}$

with a thin vinyl film to reduce the possibility of the solution spreading.

Some investigators demonstrated the postnatal development of triceps surae motoneurons in the cat [11–13].

They showed that the motoneuron size reached that of the adult cats at around 2 months. The time course of size development in the ECL and ECM motoneurons was similar to that in triceps surae muscle motoneurons. However the sudden increase in motoneuron size in medial gastrocnemius motoneurons at around 3 wks [11, 12] was not observed in ECL and ECM motoneurons. The location of ECL and ECM motoneurons did not change postnatally. Ulfhake *et al.* [13] also reported that locations of triceps surae muscle motoneurons in cats did not change postnatally.

Burke *et al.* [2, 3] showed bimodal distribution of motoneurons innervating m. medial gastrocnemius (MG) and suggested that the two peaks on the histogram of motoneuron size corresponded with gamma and alpha motoneurons in adult cats. In the MG motoneuron, Tatton and Theriault [12] reported that unimodal distribution changed to bimodal distribution on histograms at approximately 3 wks. In the present experiment, we demonstrated that histograms of ECM and ECL motoneurons sizes were not unimodal. Bimodal distribution was clearly observed in the ECL motoneuron, but not observed in ECM motoneurons. Since it has been shown that gamma-motoneurons are systematically smaller in average soma diameter than alpha-motoneurons [2, 3], we assumed that ECL motoneurons with average diameter $<30 \mu\text{m}$ were gamma-motoneurons and that those with larger diameters were alpha-motoneurons. It seems likely that both of alpha- and gamma-motoneurons are intermingled in ECM motoneurons.

In the present experiments, we observed connections between ECL and ECM motoneurons on the same side and those of ECM motoneurons on both sides at 2 wks-2 months. HRP solution should be applied intracellularly to obtain a complete visualization of dendrites in the labeled neurons [4, 13]. As shown in Fig. 3, dendritic connections could be observed after injecting a highly concentrated HRP solution (30-35%). After 2 wks of age, dendritic connections between ECL and ECM motoneurons on the same side, and those of ECM motoneurons on both sides were observed. Intensity of dendritic connections increased between 2 wks to 2 months. These motoneuronal changes appeared to be coincident with the development of controlled tail movements. Electromyograms recorded from ECL or ECM muscles on both side show (data not shown) that reciprocal activity between left and right tail muscles is absent from birth to 1 wk of age, then appears at 2-3 wks and is fully developed at 2 months. It could be considered that the developing dendritic connections are involved in the tail movements. However, Ulfhake *et al.* [13] reported that the relative size of the dendritic territory on the transverse plane remained almost constant in the triceps surae motoneurons in the cat. Further studies using intracellular labeling are required to investigate tail muscle motoneurons.

SDH and PFK indicate the oxidative and glycolytic potential of muscles, respectively. The value of PFK/SDH was rapidly increased between 1 and 2 wks of age. Buller

et al. [1] reported that all hindlimb muscles in the newborn cat showed slow contraction and the contraction time was markedly decreased between first postnatal day and 4 wks. Mann and Salafsky [7] demonstrated that the changes in the physiological characteristics, contraction time, 1/2 relaxation time paralleled changes in oxidative and glycolytic enzymes. It could be considered that the contraction time of ECL muscle fibers was markedly decreased between the 1st postnatal day and 2 wks of age.

In the present experiments, we observed postnatal changes of motoneurons and muscle fibres. The size of motoneurons was gradually increased, while biochemical characteristics of muscle fibers were suddenly changed for 2 wks. This fact might suggest that motoneuronal developments were not paralleled with muscle fiber developments.

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