

LOCALIZATION OF OBTURATOR AND
PECTINEAL MOTONEURONS IN THE DOG

by

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INTRODUCTION

A developmental myopathy characterized by hypotrophy of Type II muscle fibers has been described in the pectineus muscle of dogs 37-118 days of age (Cardinet et al., '69). The myopathy described was suggestive of a neurogenic disease of muscle which appeared to be restricted to the pectineus muscle, since lesions were not found in other muscles of the hip. Pathological changes in intramuscular nerve branches and/or lumbar spinal cords were not observed to substantiate the neurogenic origin of the muscle lesion; however, the possibility of neuropathology was not definitely excluded in the study.

The canine pectineus muscle is innervated by a branch of the obturator nerve (Miller, Christensen, and Evans, '68). Based on gross dissection (Langley and Anderson, 1896), and antidromic stimulation (Fletcher, '70), the origin of the obturator nerve is from spinal nerves L4-L6. Motor fibers innervating the pectineus muscle have likewise been reported to arise from L4-L6 (Fletcher, '70). However, the obturator and pectineal motoneuron cell columns have not been localized within these lumbar segments of the dog.

The purpose of this study was to localize the obturator and pectineal motoneuron cell columns within the canine spinal

cord by retrograde chromatolysis, and to determine by nerve stimulation, the spinal nerves contributing innervation to the pectineus muscle. The results provide an anatomical basis for neuropathological studies in dogs affected with the developmental myopathy of the pectineus muscle.

MATERIALS AND METHODS

Chromatolytic Localization of
Obturator and Pectineal Columns

Thirteen dogs, 10 females and 3 males, were used in this study. Ages ranged from 2-12 months and body weights ranged from 4-16 kilograms.

Two experimental procedures were employed to localize the motoneuron cell columns within the spinal cord: (1) obturator neurectomy and (2) pectinectomy. All surgical procedures were carried out under aseptic conditions. Obturator neurectomies were performed through a ventral midline incision. A 1-2 cm segment of the obturator nerve was removed at a point just cranial to its passage through the obturator foramen. Severance of the pectineal nerve was accomplished by removal of the entire pectineus muscle.

Unilateral surgery was performed on 3 dogs: one unilateral obturator neurectomy and two unilateral pectinectomies. Bilateral surgery was performed on 10 dogs: one bilateral pectinectomy, one bilateral obturator neurectomy and 8 pectinectomies with contralateral obturator neurectomies.

Dogs were euthanatized at postoperative periods ranging from 7-30 days. The spinal cord was fixed in situ with 10% formalin and 1% calcium chloride at pH 7.0 by aortic perfusion for 2-3 hours. It was then removed and immersed

in fixative for an additional 36-48 hours.

Lumbar segments 1-7 were identified by their dorsal and ventral rootlet orientation and separated under a dissecting microscope. Segments were washed, dehydrated, infiltrated, and embedded in paraffin. Each segment was serially sectioned, 15 μ thick, in longitudinal orientation in all but one dog. In the latter case, the segments were serially sectioned transversely at 20 μ . Sections were stained with 0.1% Toluidine Blue O in 0.1M acetate buffer, pH 4.1.

Sections were examined with a light microscope and chromatolytic neurons recognized by their characteristic decrease in stainable cytoplasmic basophilia (Bodian and Mellors, '45). The number of chromatolytic neurons in a section was determined by counting those neurons in which the nucleolus of the nucleus was in the plane of section. The dorsal to ventral, medial to lateral, and cranial to caudal boundaries of chromatolytic neurons within the gray matter were determined. Relative measurements of location rather than absolute measurements are reported to compensate for individual spinal cord size variations. In analysis of the dorsal to ventral boundaries, the dorsal aspect of gray matter was assigned a value of 0% and the ventral aspect a value of 100%. In the analysis of the medial to lateral boundaries, the medial margin of the gray matter was assigned a value of

0% and the lateral margin a value of 100%. The length of each segment was assigned a value of 100% for determination of the cranial to caudal boundaries.

Electrophysiological Localization of the Origin of the Pectineal Nerve

Nerve stimulation was performed on the pectineal nerve of 4 female mongrel dogs over one year of age. Surgical anesthesia was induced and maintained by intravenous injection of sodium pentobarbital. The dogs were placed in dorsal recumbency while the pectineal nerve was exposed at the point of its entrance into the pectineus muscle. The nerve was positioned over silver electrodes through which the stimulus pulse was delivered. The electrodes were sheathed in flexible rubber tubing which served to hold the nerve in constant contact with the electrodes. The interelectrode distance was 4 mm.

With the stimulating electrodes secured to the pectineal nerve, the animal was placed in an upright position in a stereotaxic device. Segmental orientation was achieved by palpation of the 13 thoracic vertebra and the sacrum. A laminectomy was then performed which exposed the spinal nerves of segments L3-L7. Mineral oil was placed in the pool formed by the laminectomy to prevent desiccation of the spinal nerves.

Spinal nerves L3-L7 were identified and placed individually across silver recording electrodes while results from pectineal nerve stimulation were recorded. Spinal nerves L4-L6 were then divided and antidromically evoked responses were recorded from the ventral roots. In some cases ventral roots were further divided.

Square-wave pulses of 0.05 msec duration were delivered by a stimulator (Grass Instrument Co., Model S4) and stimulus isolation unit (Grass Instrument Co., Model SIU-4). The intensity of the stimulus was supramaximal as determined by the twitch elicited in the pectineus muscle. The stimulus artifact and evoked response were amplified (Grass Instrument Co., Model DP9), displayed on a storage oscilloscope (Tektronix, Inc., Model 564), and photographed with an oscilloscope camera (Tektronix, Inc., Model C-12).

RESULTS

Chromatolytic Localization of Obturator and Pectineal Columns

Resection of the obturator and pectineal nerves resulted in variability of the chromatolytic reaction among motoneurons (Fig. 1), i.e., severely chromatolytic neurons with a marked decrease in stainable cytoplasmic basophilia were observed in the same section with chromatolytic neurons that had only a slight decrease in stainable cytoplasmic basophilia. The chromatolytic reaction varied in the postoperative time required for it to occur. Chromatolysis was observed 7-29 days following obturator neurectomy (table 1), but not until 20-30 days following pectinectomy (table 3).

Chromatolytic motoneurons following obturator neurectomy were limited to lumbar segments 4-6 with the largest number present in L5 (table 1). The obturator column was largest in L5 since it contained the greatest cranial-caudal, dorso-ventral and medial to lateral boundaries for chromatolytic neurons (tables 1,2). The cranial-caudal position of the obturator column in lumbar segments 4-6 varied; as the cranial boundary extended more anteriorly into L4 there was a corresponding decrease in the caudal extension into L6. Dogs 8, 9, and 11 showed such prefixation (table 1). Conversely, the more caudal the position of the cranial boundary in L4 the

greater the extension of the column into L6 posteriorly. Dogs 1, 5, and 6 showed such postfixation (table 1). The remainder of the dogs (4, 10, 12, and 13) contained intermediate cranial-caudal boundaries and were examples of median fixation (table 1). The obturator column within segments L4-L6 was located slightly lateral to the middle of the ventral horn (Figs. 3, 5, 6). The dorso-ventral and medial to lateral boundaries of the obturator column are presented in table 2.

Chromatolysis of motoneurons following pectinectomy was limited to spinal segments L4 and L5 (table 3). The cranial to caudal boundaries of the pectineal column were confined within those determined for the obturator column in lumbar segments 4 and 5. Various degrees of cranial to caudal fixation were observed in the pectineal column. Dogs 8 and 9 contained prefixed columns and dogs 7 and 5 contained post-fixed columns (table 3). Median fixed pectineal columns were found in dogs 4, 10, and 13 (table 3). Dogs that underwent dual operations and contained prefixed obturator columns also showed prefixed pectineal columns on the opposite side, i.e. dogs 8 and 9 (tables 1 and 3). Likewise, postfixation was observed in dog number 5 for both columns and median fixation in dogs number 4, 10, and 13 for both columns.

The dorsoventral and medial to lateral boundaries for

the pectineal column were found to be within the boundaries determined for the obturator column with the exception of the medial boundary in L4 (tables 2 and 4). However, this difference in the mean medial boundaries for the obturator and pectineal columns in L4 was not statistically significant (Student's t test, $P > 0.05$).

From the mean boundaries determined for the pectineal column, its position is illustrated in transverse sections (Figs. 2,4). Based on the boundaries determined for the pectineal and obturator columns their position in the spinal cord is illustrated in a three dimensional reconstruction (Fig. 7).

Electrophysiological Localization of the Origin of the Pectineal Nerve

Pectineal nerve stimulation evoked electrical responses in L4 and L5 spinal nerves in all 4 dogs. In one dog an evoked response was recorded from spinal nerve L6. In three dogs with evoked responses limited to L4 and L5 spinal nerves it was possible to also record from the ventral roots following antidromic stimulation. Under these conditions the evoked responses were also limited to L4 and L5. In the one dog where an evoked response was recorded from spinal nerve L6, technical difficulties prevented recording from the ventral root. In one dog it was possible to further subdivide the

L6 ventral root and record following antidromic stimulation.

In this instance no evoked responses were recorded. Examples of evoked responses in a L5 ventral root following antidromic stimulation is presented in figure 8.

TABLE 1

Number and segmental location of chromatolytic neurons following obturator neurectomy

Dog Number	Side of Neurectomy	Days Post Neurectomy	No. of Chromatolytic Neurons			Segmental Location of Chromatolytic Neurons		
			L4	L5	L6	Total	Caudal Percent of L4	Cranial Percent of L6
1	Left	7	10	114	90	214	20	81
6	Left	7	3	253	305	561	30	84
11	Left	13	121	282	54	457	70	39
12	Left	13	53	260	320	633	48	88
12	Right	13	63	241	225	529	60	89
4	Right	14	138	702	565	1405	41	96
13	Left	20	164	443	315	922	41	87
10	Right	20	163	354	207	724	44	50
8	Left	29	139	615	145	899	85	49
9	Right	29	239	521	132	892	91	45
5	Left	29	15	312	363	690	31	93
Mean			101	372	247	720	51	73
Std. Dev.			73	170	140	296	22	21

TABLE 3

Number and segmental location of chromatolytic neurons following pectinectomy

Dog Number	Side of Neurectomy	Days Post Pectinectomy	No. of Chromatolytic Neurons			Segmental Location of Chromatolytic Neurons		
			14	15	16	Total	Caudal Percent of 14	Cranial Percent of 16
6	Right	7	-	-	-	-	-	-
3	Left	13	-	-	-	-	-	-
2	Left	13	-	-	-	-	-	-
11	Right	13	-	-	-	-	-	-
13	Right	20	13	5	-	18	29	49
10	Left	20	36	15	-	51	42	27
9	Left	29	41	9	-	50	88	51
8	Right	29	84	13	-	97	80	32
7	Left	29	7	75	-	82	11	78
7	Right	29	3	82	-	85	21	92
5	Right	29	7	58	-	65	17	90
4	Left	30	25	31	-	56	45	49
Mean			27	36		63	42	58
Std. Dev.			25	29		23	27	24

TABLE 4

The dorsoventral and medial to lateral boundaries of chromatolytic neurons in the pectineus column

Dog Number	<u>Dorsoventral¹ Boundaries of Chromatolysis</u>				<u>Medial to Lateral² Boundaries of Chromatolysis</u>							
	<u>Segment I4</u>		<u>Segment I5</u>		<u>Segment I4</u>		<u>Segment I5</u>					
	<u>Dorsal</u>	<u>Ventral</u>	<u>Dorsal</u>	<u>Ventral</u>	<u>Medial</u>	<u>Lateral</u>	<u>Medial</u>	<u>Lateral</u>				
13	73	89	78	88	-	-	60	89	48	73	-	-
10	74	85	64	75	-	-	48	70	50	60	-	-
9	70	81	58	67	-	-	54	80	43	67	-	-
8	76	90	76	82	-	-	50	75	45	73	-	-
7	84	87	59	76	-	-	47	80	52	80	-	-
7	84	87	59	79	-	-	60	78	47	80	-	-
5	77	82	58	80	-	-	28	39	56	75	-	-
4	76	85	74	82	-	-	48	78	47	67	-	-
Mean	77	86	66	79			49	74	48	72		
Std. Dev.	5	3	8	6			9	14	4	6		

¹Values for the dorsoventral boundaries are relative distances where the entire dorsoventral depth of the gray matter in a hemisection is taken to be 100; 0 represents the dorsal margin of the dorsal gray and 100 represents the ventral margin of the ventral gray matter.

²Values for the medial to lateral boundaries are relative distances where the entire medial to lateral width of gray matter in a hemisection is taken to be 100; 0 represents the medial margin of gray matter and 100 represents the lateral margin of gray matter.

DISCUSSION

The chromatolytic reactions observed in this study were similar to those previously described following peripheral nerve resection (Barr and Hamilton, '48; Romanes, '51; Cole, '68). The variation in the time sequence of chromatolysis observed between obturator neurectomies and pectineal neurectomies might be a function of the proximity of the resection to the central nervous system. More rapid onset of chromatolysis is observed when the axon is interrupted close to the cell body as opposed to far distally in a limb (Bodian and Mellors, '45). The obturator neurectomies were more proximal than the pectineal neurectomies. Also, less variable results have been observed following damage to large nerves as compared to damage to individual nerves supplying a muscle (Romanes, '51). The numbers of chromatolytic neurons observed following obturator neurectomies are similar to those reported for the cat (Romanes, '51).

The results of this study localize the obturator motoneuron column of the dog within spinal segments L4-L6. This finding is consistent with reports that the obturator nerve is derived from spinal nerves L4-L6 based on gross dissection (Langley and Anderson, 1896) and antidromic stimulation studies (Fletcher, '70). Degrees of cranial-caudal fixation were observed; however, shifts cranial or caudal were confined within

segments L4-L6.

The patterns of motoneuron columns within the dog are described as similar to those of the cat (Elliott, '44). The position of the obturator column in the ventral horn of the dog roughly corresponds to the position described as Column 3 for the cat (Romanes, '51) which would be located within lamina IX (Rexed, '64). However, the obturator columns of the dog and cat differ in their segmental location since the obturator of the cat is contained within segments L5 and L6.

The localization of the pectineal motoneuron column within the limits of the obturator motoneuron column is consistent with the derivation of the pectineal nerve as a branch of the obturator nerve (Miller, Christensen, and Evans, '68). The pectineal nerve has been described as being derived from spinal nerves L4-L6 where antidromic stimulation techniques have been employed (Fletcher, '70). In the present study, localization of the pectineal motoneuron column judged by chromatolysis was confined to spinal segments L4 and L5. Also, the derivation of the pectineal nerve judged by antidromic stimulation was confined to ventral roots L4 and L5. In the one instance where an evoked response was observed in spinal nerve L6, it was not possible to record from the ventral root to determine if the response was mediated by conduction via afferent or efferent fibers. With this possible exception, the results obtained by

both methods of analysis complemented each other, and it seems likely that in most instances the derivation of the pectineal nerve in the dog is from spinal segments and nerves L4 and L5. The pectineal motoneuron column has not been localized in other species.

The results of this study localize within the spinal cord the motoneurons of the obturator and pectineal nerves and provide an anatomical basis for investigations of motoneurons in the developmental myopathy affecting the pectineus muscle of dogs.

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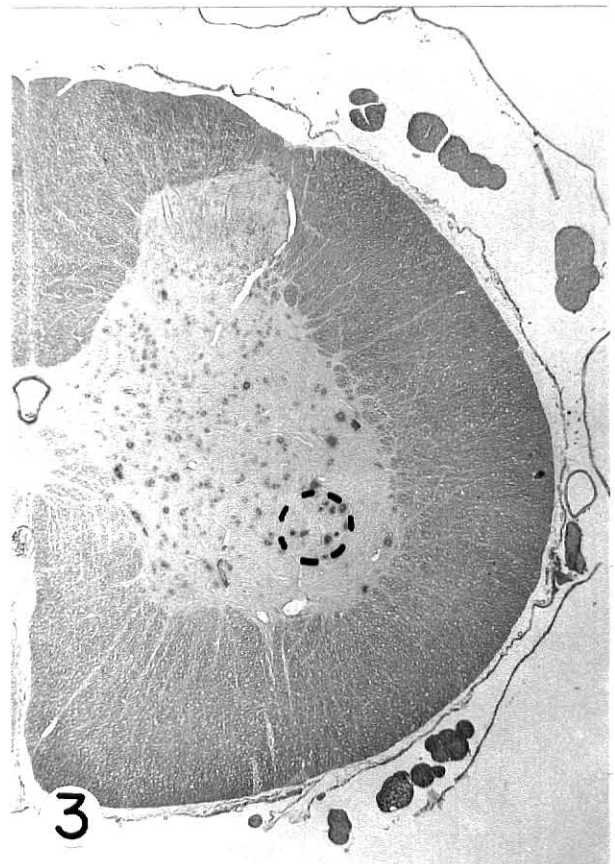
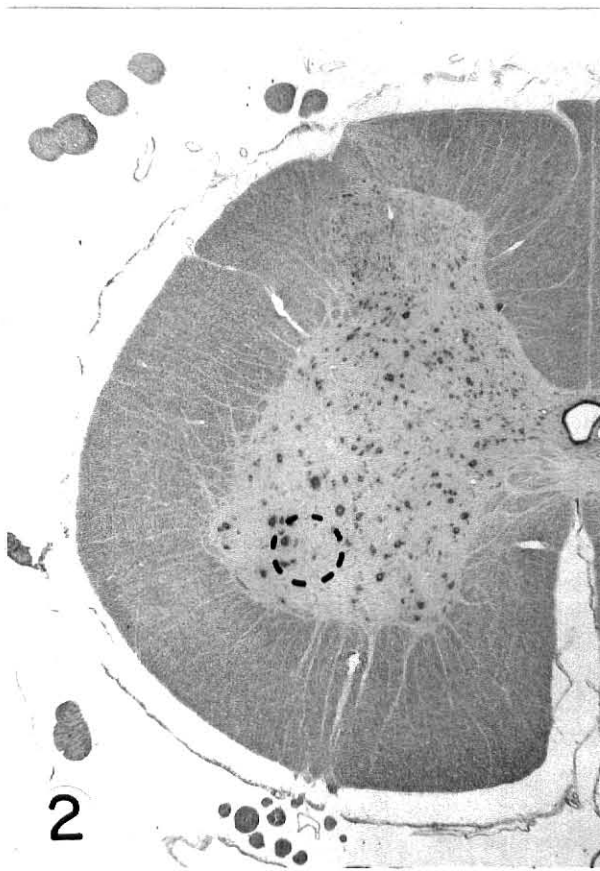
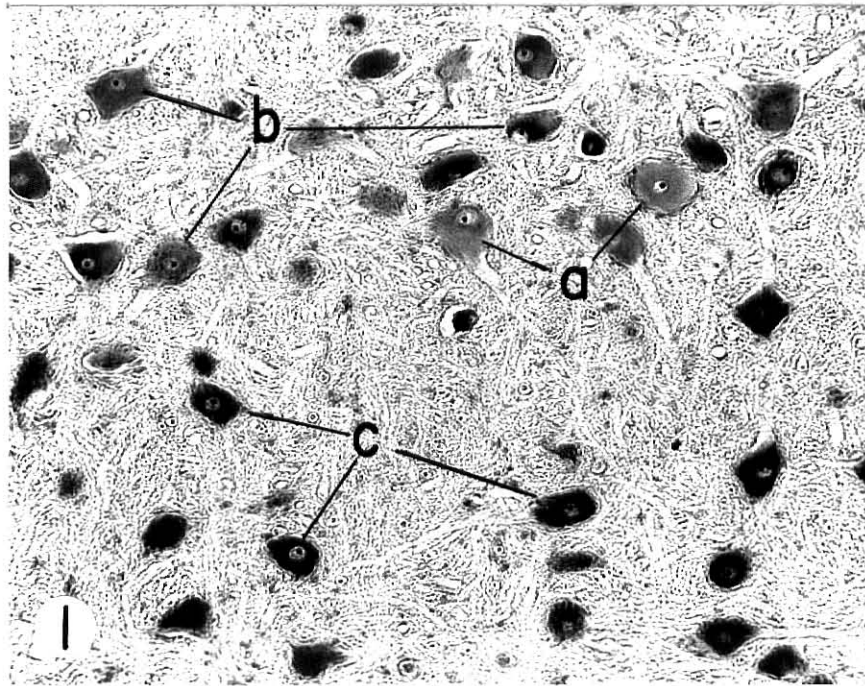
Finally, recognition is due Carolyn Oswalt for the typing of this thesis.

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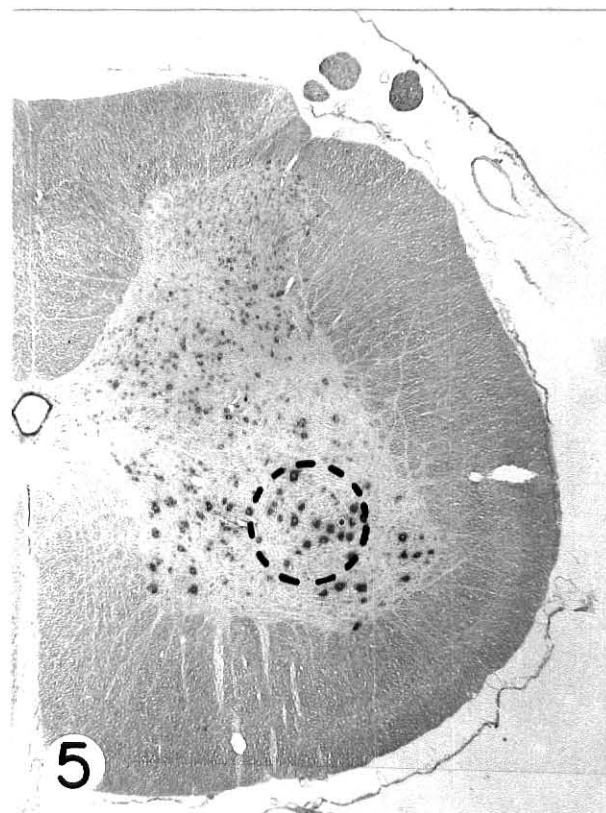
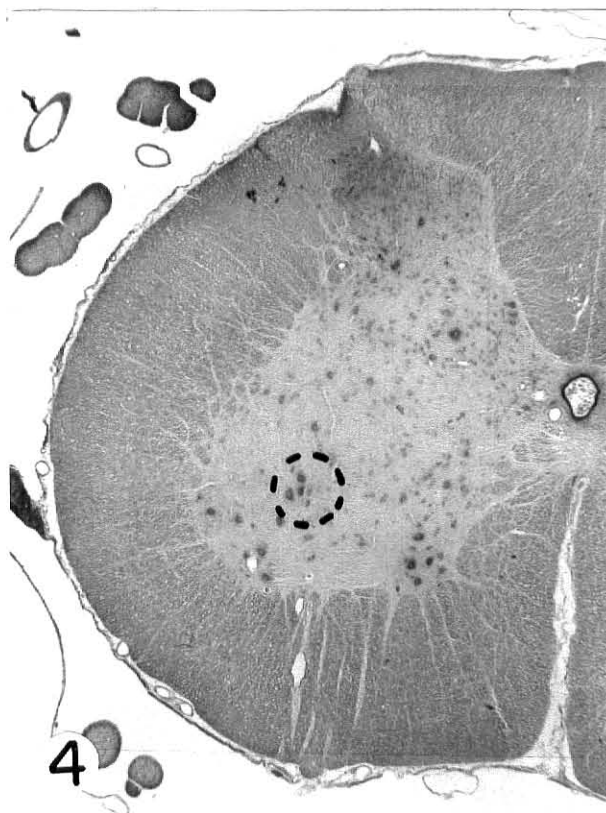
EXPLANATION OF FIGURES

- Fig. 1. Chromatolytic reaction of motoneurons 14 days after obturator neurectomy (Dog 4). Variations in chromatolysis varied from severe (a) to moderate (b). These may be compared to normochromic motoneurons (c). x 115
- Fig. 2. Boundaries of pectineal column; illustration based on mean values determined for dorsoventral and medial to lateral boundaries. Transverse section from caudal 25% of L4. x 25
- Fig. 3. Boundaries of obturator column; illustration based on mean values determined for dorsoventral and medial to lateral boundaries. Transverse section from caudal 10% of L4. x 25



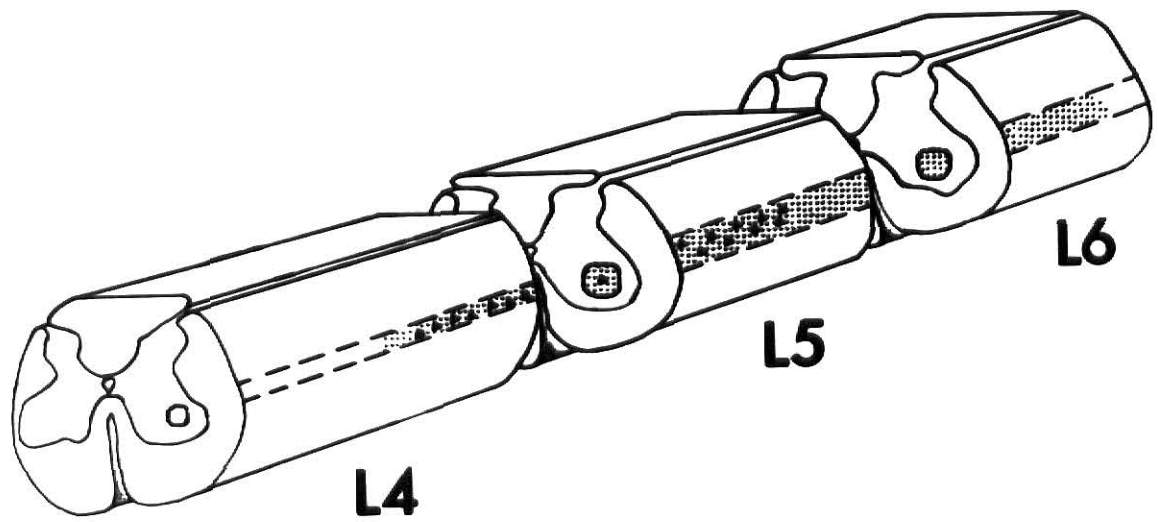
EXPLANATION OF FIGURES

- Fig. 4. Boundaries of pectineal column. Illustration based on mean values determined for dorsoventral and medial to lateral boundaries. Transverse section from cranial 10% of L5. x 25
- Fig. 5. Boundaries of obturator column. Illustration based on mean values determined for dorsoventral and medial to lateral boundaries. Transverse section from cranial 15% of L5. x 25
- Fig. 6. Boundaries of obturator column. Illustration based on mean values determined for dorsoventral and medial to lateral boundaries. Transverse section from cranial 25% of L6. x 25

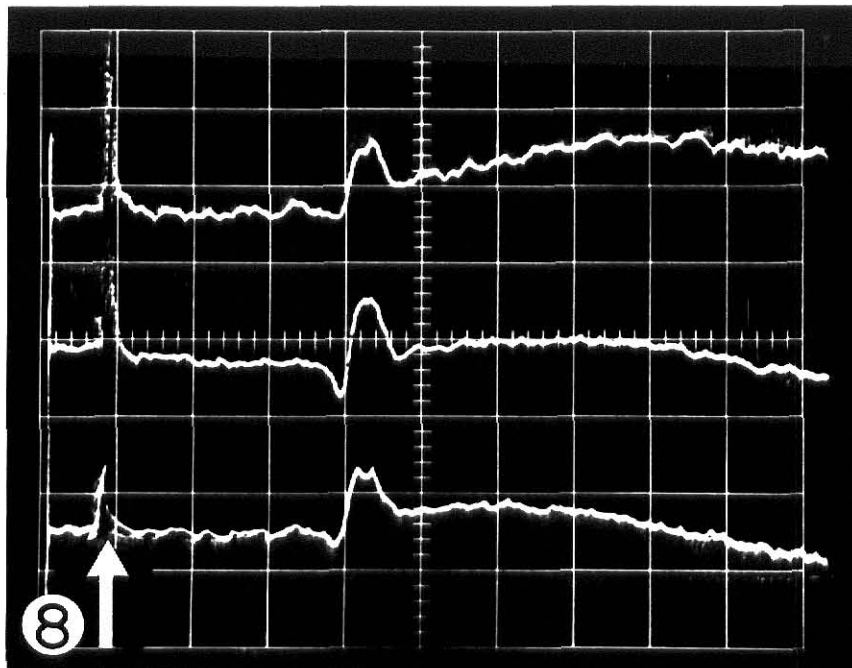


EXPLANATION OF FIGURES

- Fig. 7. Three dimensional reconstruction of obturator and pectineal motoneuron columns. Extent of obturator column is denoted by stippling while pectineal column is denoted by symbol (\blacktriangle). The obturator column was found to extend between the caudal 50% of L4, all of L5, and the cranial 75% of L6. The pectineal column was found to extend between the caudal 40% of L4 and cranial 60% of L5.
- Fig. 8. Oscilloscope recordings of evoked responses from ventral root L5 following antidromic stimulation of the pectineal nerve. The recording is typical of the evoked responses observed in L4 and L5 following antidromic stimulation. The evoked responses were repeatable as evidenced by the three evoked responses presented. The stimulus artifact is denoted by the arrow. Large vertical divisions equal 30 uv/div. Large horizontal divisions equal 0.5 msec./div.



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LOCALIZATION OF OBTURATOR AND
PECTINEAL MOTONEURONS IN THE DOG

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AN ABSTRACT OF A MASTER'S THESIS

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The obturator and pectineal motoneuron columns were localized in the canine spinal cord by analysis of retrograde chromatolysis following resection of the obturator and pectineal nerves. The obturator column was localized in spinal segments L4-L6 while the pectineal column was limited to segments L4 and L5. The boundaries of both columns within the ventral horns were determined and the pectineal column was localized within the boundaries determined for the obturator column. The axons of motoneurons of the pectineal nerve were determined to be derived from ventral roots of spinal nerves L4 and L5 by antidromic stimulation.