

Participation of the Intercostal Nerves to the Innervation of the Diaphragm Muscle in *Cavia porcellus*

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Abstract

Introduction The diaphragm is the leading respiratory muscle. It is innervated mainly by the diaphragmatic nerve, and, in some species, by the delicate fibers of the intercostal nerves. In guinea pigs, there is no description of these anatomic structures that innervate this important muscle. This study aimed to analyze the participation of the intercostal nerves to the innervations of the diaphragm of guinea pigs of both sexes.

Materials and Methods We studied 40 guinea pigs (*Cavia porcellus*) of both sexes. We fixed and dissected the diaphragm of the specimens used in the experiment to assess the path of the intercostal nerves in both the body antimeres.

Results The diaphragm was innervated by the intercostal nerve pairs 6 through 12, and, less frequently, by the 8th nerve (38/40 = 95%), followed by the 7th (36/40 = 90%) and subsequently by the 9th (32/40 = 80%). The 12th nerve presented the lowest frequency (2/20 = 10%) in both genders. All nerve pairs displayed similar occurrence compared with the gender and the antimeric disposition. The only exception was the 9th nerve, which presented a significant variation of the occurrence, both in relation to gender and antimeric disposition. From a statistic point of view, all nerves were independent. We observed no correlation between the gender and their position.

Conclusions We shall conclude that the diaphragm of guinea pigs is innervated by the 6th through 12th pairs of intercostal nerves, with the 7th, 8th, and 9th being the primary providers. There is no interference of the variables gender or antimeric disposition on the behavior of the intercostal nerves of guinea pigs as refers to their origin and participation to the innervations of the diaphragm.

Keywords

- ▶ guinea pig
- ▶ diaphragmatic muscle
- ▶ innervation

Introduction

Breathing is the only form to provide oxygen to human beings and animals. As it enters into the organism, the oxygen combines with most of the nutrients before entering the cells and producing the energy that is crucial for

the performance of physiologic processes required for survival. The muscles enrolled in the respiratory process are the diaphragm, the scalene, and the intercostal muscles.¹ The diaphragm is deemed to be only one and essential respiratory muscle,² contributing from 60 to 80% of the respiratory process,³ as it is the main muscle

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involved in the inspiration process. The diaphragm is not only enrolled in the act of breathing. It is also crucial to assist the abdominal muscles during vomiting, coughing, urination, sneezing, defecation, and labor.⁴ Almeida et al (2008)⁵ describe the intercostal nerves, mainly those from the 7th to the 9th, as primary efferent pathways for some of these actions or reflexes.

The diaphragm receives motor nerves mainly, or solely from the phrenic nerves.^{6–13} Even the same, in some species, there is a participation also from branches derived from the intercostal nerves.^{5,14–19} There are 12 pairs of intercostal nerves. They derive from the ventral branches of the thoracic nerves, do not form plexus and distribute to the walls of the thorax and the abdomen. Almost all nerves lie in the intercostal space (therefore they are denominated “intercostal nerves”). The 12th intercostal nerve is named *subcostal*, as it lies under (behind) the last rib.²⁰

Guinea pig (*Cavia porcellus*) is a primary species for the science. It is frequently used for studies on the anatomy and physiology that shall be applied to the humans and other species.^{21–23} Even with the diaphragm being responsible for such an essential function as breathing, there are no studies on the role of the intercostal nerves to its innervation in guinea pigs.

This study aimed to assess the participation of the intercostal nerves to the innervation of the diaphragm in guinea pigs *Cavia porcellus* of both genders and to describe the collateral innervation of this organ by the intercostal nerves.

Materials and Methods

In this study, 40 healthy adult male ($n = 20$) and female ($n = 20$) guinea pigs *Cavia porcellus* (Linnaeus, 1758) were used. The animals came from the central laboratory animal house of the Universidade Federal do Vale do São Francisco (UNIVASF, in the Portuguese acronym). This study was approved and certified by the ethical committee for the use of animals of the UNIVASF (CEUA, in the Portuguese acronym, protocol number 0012/160512).

The specimens were transported to the laboratory of domestic and wild animal anatomy of the UNIVASF (LAADS,

in the Portuguese acronym), where they were euthanized (40 mg/kg of 5% ketamine; 10 mg/kg of 2% xylazine intraperitoneally to induce anesthesia, followed by 5 ml of 10% potassium chloride, intraperitoneally). As a criterion for the enrollment, the animals should not present any anomaly or pathology in the thoracic or abdominal region.

After the euthanasia of the guinea pigs, the left common carotid artery was cannulated to perfuse a 10% water solution of formaldehyde using 20 mL syringes and 25×7 needles. After this, the anatomic pieces were dipped in 10% formaldehyde solution for 72 hours to fix the tissues. The costochondral articulations were incised to fold them to the exterior from the fifth rib on, in both antimeres. Every intercostal nerve was dissected. All the ramifications were conserved, especially those directed to the diaphragm. After their identification, the ramifications of the intercostal nerves were photographed and compared, to assess their position in the antimeres and the sex of the animal.

Statistical analysis was performed using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, USA), considering the chi-square test and phi coefficient, and analyzing the independence between the gender (female, or male) and the position (left or right); the independence level between the position and the intercostal nerves and the occurrence of the participation of these nerves to the innervation of the diaphragm. A 5% significance level was adopted ($p < 0.05$).

Results

As described in ►Table 1, the ramifications derived from the intercostal nerve pairs 6 to 12 innervate the diaphragm of guinea pigs. According to the frequency of occurrence, the intercostal nerves were classified in four groups: A group (average) represented by the nerves 6 and 10; B group (high) represented by the nerves 7, 8, and 9; C group (intermediate) represented by the 11th nerve, and D group (low), represented by the nerve 12. We observed no significant difference among the nerves classified in the same group of occurrences.

Table 1 Frequency and percentage of occurrence of the intercostal nerves in the innervation of the diaphragm of guinea pigs (*Cavia porcellus*), according to the antimeres and the gender—Petrópolis (PE), 2018

Nerve	VI		VII		VIII		IX		X		XI		XII	
Gender	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Females ($N = 20$)														
FR	12	13	16	18	18	18	17	14	10	11	4	5	1	2
%	24.5	26.5	22.9	25.7	24.3	24.3	27.0	22.2	24.4	26.8	26.7	33.3	14.3	28.6
Males ($N = 20$)														
FR	11	13	18	18	20	18	17	15	10	10	3	3	2	2
%	22.4	26.5	25.7	25.7	27	24.3	27.0	23.8	24.4	24.4	20	20	28.5	28.5
Total	23	26	34	36	38	36	34	29	20	21	7	8	3	4
%	46.9	53.1	48.6	51.4	47.4	48.6	54	46	48.8	51.2	46.7	53.3	42.9	57.1

Abbreviations: F, female; FR, frequency; L, left antimeres; M, male; R, right antimeres.
%= percentage

The nerve pairs of the A group, 6 and 10, displayed similar occurrence ($p > 0.05$) according to both the gender and the antimeric disposition. Both nerves presented 65% of occurrence in males, and females.

Nerves pairs of the B group 7, 8, and 9 displayed similar occurrence according to both the gender and the antimeric disposition. The 7th nerve was present in 90% (18/20) of the specimens, both male, and female. The 8th nerve too was present in 90% (18/20) of the females, and 100% (20/20) of the males. The 9th nerve was visible in 85% (17/20) of the females and males. **Fig. 1** shows the innervation of the diaphragm by the 7th intercostal nerve.

The nerve of the C Group, 11th, showed a similar occurrence ($p > 0.05$) as concerning the gender and the antimeric disposition, in agreement with the other nerves. Even if the participation of this nerve in the innervation of the diaphragm was not relevant, it was present in 55% (11/20) of the female, and 50% of the male subjects.

The nerve of the D Group, 12th, showed a similar occurrence ($p > 0.05$) as concerning the gender and the antimeric disposition, in agreement with the other nerves. Its participation to the innervation of the diaphragm was the lowest. It was only present in 10% (2/20) of the female, and male subjects.

The Chi-square test over 0.05, with a value of 0.82, corresponding to 8%, and Phi-coefficient of -0.026 statistically confirm the independence and the absence of any correlation between the gender and the position among all the nerves that have been studied.

When analyzing the relationship between gender and the occurrence of the nerves either in the left, or in the right antimeric, we observed that in females the occurrence was more frequent ($p > 0.05$) in the left antimeric (51% = 81/159), than in the right (49% = 78/159). On the other hand, in males, the occurrence was more common in the right antimeric (51% = 81/160) than in the left (49% = 79/160). Among the females, all intercostal nerves except the 8th and 9th were observed more frequently in the left antimeric. The 8th nerve

displayed the same occurrence in both antimeres and the 9th was more frequently observed in the right antimeric. Among the males, the nerves 8 and 9 displayed more frequent occurrence in the right antimeric. The nerves 7, 10, 11, and 12 displayed the same occurrence in both antimeres. The nerve 11 was more frequent in the left antimeric than in the right one (**Table 1**).

Discussion

The results of this study highlight the importance of the intercostal nerves as main efferent ways for the breathing in guinea pigs, not just as motor fibers. These findings disagree with the results of previous studies, which claimed that the phrenic nerve was the main, and probably the only source of innervation of the diaphragm in some mammalian species.^{6–13} On the other hand, our findings are in agreement with other studies that pointed out the contribution of the intercostal nerves to the innervation of the diaphragm.^{4,5,14–19}

We observed the innervation of the diaphragm by the intercostal nerves 6 to 12, mainly by the 8th nerve. Rosenbluth et al (1961),⁴ even if they observed the participation of the intercostal nerves 4 to 10, observed that the major intercostal nerve acting in the innervation of the diaphragm was the 8th nerve. In dogs, MELO et al (1999)²⁴ observed the innervation of the diaphragm by the intercostal nerve pairs 8 to 12 in 93.54% of cats and dogs. Faria et al (2011)¹⁶ observed the central innervation of the diaphragm by the intercostal nerves 9 through 11, but the possible participation of the nerve fibers from the intercostal nerves 7, 8, and 12. Oliveira et al (2001)²⁵ observed that the diaphragm of hybrids of bovines and zebus received the innervation of the intercostal nerves 7 through 12 in 50% of the animals; from the intercostal nerves 6 through 12 in 33.3%; from the left antimeric of the 6th intercostal nerve in 13.3% of the animals, and from the right antimeric of the 7th nerve in 3.3% of the animals. Almeida et al (2008)⁵ observed in bovines from the Santa Inês breed, the contribution to the innervation of the diaphragm of the intercostal nerves 8 to 12

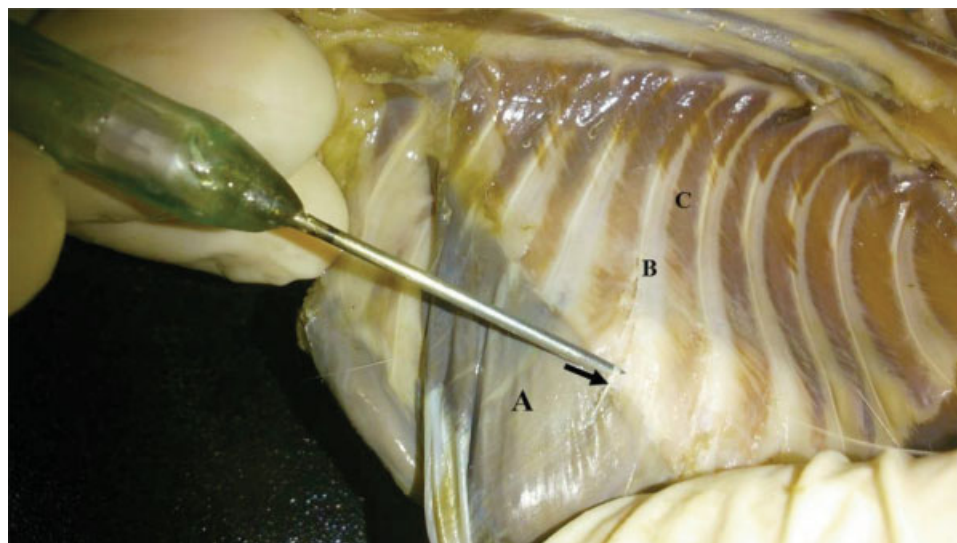


Fig. 1 Photograph highlighting the participation of the VII intercostal nerve (arrow) to the innervation of the diaphragm (A) of guinea pigs (*Cavia porcellus*). In (B), the seventh rib is visible and in (C), the intercostal muscle—Petroliana (PE), 2018.

in 63.33% of the animals, and of the pairs 9 to 12 in 26.68% of the animals, in both antimeres. In agreement with our results, these authors also did not observe any difference in the participation of the intercostal nerves to the innervation of the diaphragm neither according to the gender nor the antimeric disposition.

In this study, we observed little participation of the intercostal nerve 12 (10%) to the innervation of the diaphragm in both genders, in disagreement with the study of Melo et al (1999),²⁴ who observed that the 7th pair of nerves innervated the diaphragm in only 3.22% of the cases.

Conclusions

We shall conclude that the diaphragm of guinea pigs is innervated by the 6th through 12th pairs of intercostal nerves, with the 7th, 8th, and 9th being the primary providers. There is no interference of the variables gender or antimeric disposition on the behavior of the intercostal nerves of guinea pigs as refers to their origin and participation to the innervation of the diaphragm.

Conflicts of Interest

The authors have no conflicts of interest to report.

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