Analysis of Normal and Denerved Laryngeal Vocalization in Guinea Pigs (*Cavia porcellus*)

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Summary. Paralysis of the left vocal chord is frequent in human clinical practice; because of its anatomic similarity with human, the guinea pig might be a suitable biological model to analyze the phoniatric behavior in denerved animals. Forty newborn guinea pigs were used (20 control and 20 experimental); an incision was made in the ventricular region with the animals under general anesthesia over the middle line of the neck, until the lower left laryngeal nerve was found, the same was secured with alligator clips so that afterward a two-part dissection could be performed and the middle section could be removed (1 cm) from the nerve endings (distal and proximal) before they were separated from the laryngeal structure. After recovery from surgery, vocal emissions were recorded in solitary for 6 minutes. The animals that had nerves removed showed an increase in fundamental vocalization frequency compared with the controls. *F* test was carried out ($P = 0.05$) and no significant difference was found. When analyzing functional recovery, we found that the guinea pigs compensated vocal emissions at 20 days. With regard to the unilateral paralysis, the motility was frequently compensated by the healthy vocal chord, improving voice emission, and loss of air inhalation.
Key Words: Laryngeal denervation–Vocalizations–Vocal behavior–Guinea pigs.

INTRODUCTION

The larynx is fundamental to vocalize, is anatomically related to structures such as the esophagus, trachea, hyoids, thyroid and parathyroid glands, lymph nodes, and large blood vessels.[1,2]

Phoniatric pathology has revealed problems associated with laryngeal nerves, such as unilateral or bilateral paralysis of the vocal chords, frequently caused by iatrogenic lesions produced by recurring laryngeal nerves found in neck surgery.[3–5] In addition, another cause involving one or two larynx nerves is the bilateral paralysis of the vocal chords because of iatrogenia or neck tumors,[6,7] resulting in respiratory problems, such as inspiratory dyspnea.[8]

Lesion to the nerves is also common in tracheotomy, and thyroid and parathyroid surgeries.[2,4,9,10]

It is well known that the human larynx has important functions such as preventing solids or liquids from passing to the respiratory tract, regulation of air pressure in breathing, and generating sounds (the last being what is analyzed in this work).[11] The diversity and complexity of this function have to be coordinated by a complex nervous system driven primarily by the vagus nerve and a series of motor and sensory fibers. The motor fibers send specific information to the intrinsic laryngeal muscles so that they can carry out their function. The sensory fibers transport the information to the central nervous system and the autonomic fibers control the vasomotor reaction and secretion from the laryngeal glands.[11]

Shindo et al[12] analyzed a different process while removing the laryngeal nerve, to become familiar with the functional recuperation of various organs where the nerve had been removed. The anatomy of the superior laryngeal nerve (SLN) from the guinea pig is very similar to the one from the human, the SLN emerges from the ganglion nodosa that later divides and branches off internally and externally, and the inferior laryngeal nerve (ILN) or recurrent that is joined to the larynx and is called the internal larynx nerve deriving from the fore and after branch.[1]

The guinea pig (*Cavia porcellus*) is neurologically mature at birth, it is born with its eyes open and with fur; it has functional sight and audition from birth.[13,14] It is supposed that the emission vocalizations can be attributed to stress caused by the absence of sight, smell, and sounds of the mother. The production vocalizations in the newborn cause the mother to go into protection behavior. Some ontogeny studies have been made on voice behavior in guinea pigs.[15,16]

The guinea pig is an ideal model for audiological and phonological research[17] because of the anatomical and physiological characteristics of the ear and the audible vocalizations in the human audible range.[17–21]

The emission audible vocalization in the guinea pig is fundamental from birth to early age for auditive memory development, compared with other rodents that are born immature.[18,22,23] The guinea pig normally makes very characteristic vocalizations when separated from the mother, which are called "isolation calls"; these cries may be induced by separating newborns in new environments from their dams.[24–27] As mentioned before, these rodents emit acoustic sounds within the range of human audition. Berryman (1976) has described 11 different types of audible vocalizations in these species.[15]

Paralysis of the left vocal chord is frequent in clinical practice because the recurrent laryngeal nerve is long and surrounds the ascending aorta, for which it is more vulnerable because of medical intervention and the highly sensitive anatomical position.[17,28] The aim of this study was to analyze longitudinally the guinea pig vocalizations with denerved laryngeal surgery at the birth.

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MATERIAL AND METHODS

Laboratory animals used

Forty newborn male guinea pigs (*Cavia porcellus*) were used; animals were later divided into two groups: (1) 20 males were used as a control group (CG) and (2) 20 males were used for the experimental group (EG). The rodents were selected from 20 guinea pig litters; the colonies were formed by the mother and the litters. The average number of pups per litter was four, of which two animals were chosen for the study, one for the EG and the other for the CG. Two males with higher weight and similar size were selected from each litter so that the groups would be equal. The average weight was 110 ± 7 g.

Housing and husbandry

Animals were kept in the biotery at the National Institute for Rehabilitation (NIR), in boxes measuring 70 cm × 50 cm × 33 cm, under a light-blackout period of 12 hours, starting with a light period at 8:00 AM, the temperature was maintained at 22 ± 2°C. Oloblast compressed corn-based bedding was used. A commercial diet was provided using Guinea Pig Diet 5025 *ad libitum* and the diet was complemented with water and fresh vegetables.

Pregnant females gave birth in separate cages: at birth, rodents were given a day to physically stabilize and the surgery was programmed. The animals were treated according to the manual for use and care of experimental animals,[29] with the protocol number 150/04, sanctioned by the scientific committee and ethics of the NIR in Mexico. All studies were carried out under strict adherence to the use and care of laboratory animals and the Mexican official regulation (NOM-062-ZOO-1999).

Surgical procedure

After anesthesia with ketamine (70 mg/kg) and xylazine hydrochloride (0.5 mg/kg), animals were prepared for the surgical procedure, cutting the hair, shaving, and disinfecting the neck area with benzalkonium chloride. The surgical procedure was carried out using a CARL ZEISS 6615 microscope (Germany, K90/27), with a 16× lens.[30]

To locate the left larynx nerve, the animal was placed on its back on the surgery table and an incision was made in the middle of the neck at the throat cutting along the cutaneous and the subcutaneous planes until locating the thymus, moving the same to the right side to identify the left sternohyoideus and sternothyroid muscles, which were moved to the side exposing the inferior horn of thyroid cartilage and the carotid artery, this way managing to identify the ILN which was held with an alligator clip and later cut into two parts, the middle section (approximately 1 cm) was removed; the objective of cutting this segment is to avoid adhesion to the laryngeal structures and to avoid formation of a possible anastomosis and as a consequence the effects of laryngeal synkinesis, therefore modifying the laryngeal functions (Figures 1 and 2), although electrocoagulation can also prevent this process.[31]

Audio recordings

Recordings of the vocalizations were made inside a sound chamber from Monday to Friday, leaving Saturday and Sunday free and reestablishing recording experiment the following Monday, which took place until 20 days of recording had been completed. For the purpose of recording, a digital Sony model IC Recorder ICD-B16 (Sony Corp., Japan) was placed inside the recording chamber.

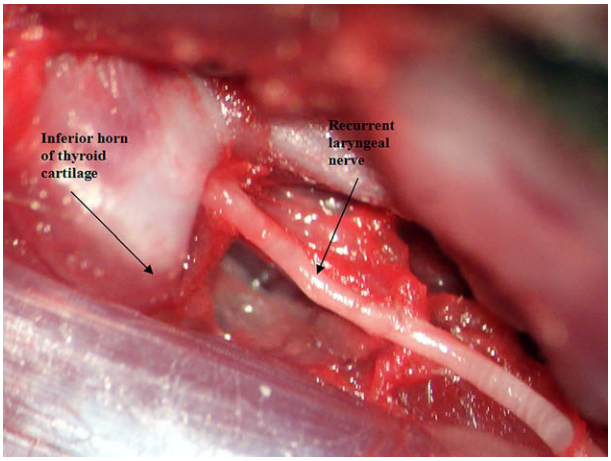

FIGURE 1. The recurrent laryngeal nerve and the inferior horn of the thyroid cartilage.

The recordings were made as follows: the offspring were isolated in the recording chamber for 6 minutes, so that vocalizations that were emitted could be recorded during this time. The same process was carried out for both groups. The recording order for both groups (EG and CG) was altered each day to avoid possible conditioning of the animals. That is, the guinea pigs that had the nerve removed were recorded first and the next day the CG was recorded successively.

Data analysis

The vocalizations of each subject were analyzed daily. After the recordings were complete, the vocalizations were filtered and digitalized using a computer, with *Cool Edit 96* software, version 6, which edits and saves .WAV audio files. The sounds emitted by the guinea pigs were analyzed using spectrograms that identified the fundamental (F_0), and formants frequencies, as well as inspiration and expiration (Figures 3 and 4).

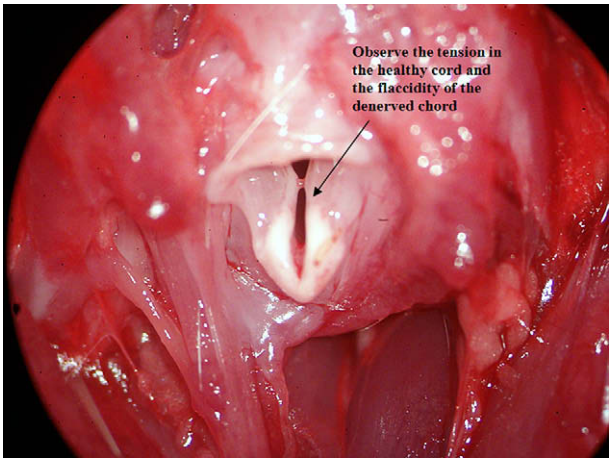

FIGURE 2. The animal's vocal chord is flaccid postsurgery; we observed that the chords are not completely closed.