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Effects of hypoglossal and facial nerve injuries on milk-suckling

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Abstract

Functional roles of the perioral anatomical structures involved in breastfeeding were examined in newborn rat pups in which the hypoglossal (XII) and facial (VII) nerves had been resected at the neonatal stage. The XII nerve controls tongue movement and is comprised of two functionally distinct branches: the medial branch related to protrusion of the tongue and the lateral branch related to its retraction. Newborn rat pups with bilateral resection of either of the XII nerve components (main trunk: XII-trunk; medial branch: XII-med; lateral branch: XII-lat) failed to suckle milk and did not survive. Unilateral XII nerve-resected neonates showed different milk-suckling capabilities, which thus resulted in differences in survival rate (XII-trunk: 38%; XII-med: 24%; XII-lat: 92%) and postnatal growth during the postnatal 3 weeks until P21. Unilateral and bilateral resections of the VII nerve innervating the buccolabial musculature produced lowered suckling capabilities and retarded postnatal growth, although all pups showed 100% survival. The results indicate a crucial role of the tongue, especially of protruding muscular elements innervated by the XII-med nerve, in breastfeeding. The results also indicate differential effects of the VII and XII nerve components on suckling capability, survival, and postnatal growth of newborn rat pups.

Keywords: Survival; Postnatal growth; Suckling capability; Cranial nerves; Tongue movement

1. Introduction

Mammals cannot survive without effective suckling after birth. There is no doubt that perioral anatomical structures play important roles in suckling. However, this issue has not been confirmed based on reliable scientific data in experimental animals. It has been speculated that the buccolabial musculature (buccinator and orbicularis oris muscles) is essential for breastfeeding by holding the mother's nipple by the action of the orbicularis oris muscle and by creating the intraoral negative pressure by the action of the buccinator muscle, both of which are controlled by the facial (VII) cranial nerve (Collins, 1995; Salmons, 1995). However, we reported recently that the buccolabial muscles play an important role in breastfeeding, but that they are not essential for the survival of newborn rat pups with bilateral VII nerve resection (Fukushima et al., 2004).

In newborn humans, cineradiographic (Arden et al., 1958) and ultrasonographic (Hayashi et al., 1997) studies have demonstrated active movement of the tongue in milk squeezing due to compression of the nipple and by changing the intraoral space to create negative pressure, indicating fundamental roles of the tongue, a free-moving intraoral muscular component, in breastfeeding. Tongue movement is controlled by the hypoglossal (XII) cranial nerve, and electrophysiological studies showed the XII nerve to be made up of two functionally distinct branches: the medial branch related to protrusion of the tongue and the lateral branch related to its retraction (Lowe, 1978; Fuller et al., 1999). Thus, the XII nerve components were suggested to play distinct functional roles in breastfeeding.

For the study of breastfeeding, it is important to quantify the amounts of suckled milk in animals in the suckling period. With the milk transfer method used generally in human babies (Mennella and Beauchamp, 1991; Prieto et al., 1996) and in newborn rats (Houpt and Epstein, 1973; Lincoln et al., 1973; Hall and Rosenblatt, 1977; Cramer and Blass, 1983), we estimated the amount of milk suckled by each pup based on the difference in body weight just before and after lactation. By setting a deprivation period for 4 h and by adequate excretion of urine just before lactation, we succeeded in quantifying the

Abbreviations: P, postnatal; VII, facial; XII, hypoglossal; XII-lat, hypoglossal lateral branch; XII-med, hypoglossal medial branch; XII-trunk, hypoglossal main trunk

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amounts of milk intake in developing rats under normal and pathological conditions.

The present study was performed to determine the functional roles of perioral anatomical structures in breastfeeding in newborn rat pups with injuries to the VII and XII nerves innervating the perioral regions. We report the effects of nerve resection on the survival and postnatal growth of newborn pups, and provide quantitative data regarding the amounts of milk intake in normal, control, and nerve-resected pups.

2. Materials and methods

2.1. Animals

Newborn Wistar rat pups (Japan SLC Inc., Hamamatsu, Shizuoka, Japan) were used in this study. Postnatal day (P) zero refers to the first 24 h after birth. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and protocols were approved by our Institutional Animal Care and Use Committee. Every effort was made to minimize animal suffering and pain as well as the number of animals used necessary to produce reliable scientific data.

A total of 523 newborn pups from 53 pregnant dams were used in the present study. After the delivery of newborn pups (P0), we observed the behavioral responses of the dam after removal and insertion of the pups. The dam immediately grouped the pups, licked them, crouched over them, and lactated them (Jirik-Babb et al., 1984; Yeo and Keverne, 1986; Kinsley et al., 1994). Before starting the experiments on P1, we first confirmed that the dam exhibited such maternal behavior on P0 and that the pups contained milk in their stomachs. In cases in which the dam did not show the maternal behavior and the pups did not contain milk in their stomachs, the animals (8 dams and 79 neonatal pups) were excluded from the experiments. In addition, in cases in which all siblings died because of the absence of the maternal behavior after surgical procedures, the animals (2 dams and 20 neonatal pups) were also excluded from the experiments. Identification of individual pups was made by small incisions of an auricle or a finger or toe tip.

2.2. Surgical procedures

Surgical manipulations of neonatal pups (P1) were performed under general anesthesia with hypothermia (−20 °C, 20 min). The XII nerve was exposed lateral to the hyoid bone, and nerve resection (about 1.5 mm in length) was made at three different sites: the main trunk (XII-trunk), medial branch (XII-med), or lateral branch (XII-lat). The VII nerve was exposed below the parotid gland, and a segment of its main trunk about 1.5 mm in length was resected. In the control pups, the XII or VII nerve was exposed and left intact. The pups were divided into 10 experimental groups: normal ($n = 25$) with no surgical procedures, control ($n = 48$) with nerve exposure only, unilateral ($n = 75$) or bilateral ($n = 31$) XII-trunk nerve injury, unilateral ($n = 80$) or bilateral ($n = 17$) XII-med nerve injury, unilateral ($n = 52$) or bilateral ($n = 19$) XII-lat nerve injury, and unilateral ($n = 39$) or bilateral ($n = 38$) VII nerve injury. To eliminate differences resulting from being born from different dams, normal, control, unilateral XII and VII nerve-resected, and bilateral XII and VII nerve-resected pups consisted of matched pairs born from the same dam. A total of 327 newborn pups from 33 pregnant dams were used for experiments to examine the survival and postnatal growth, and a total of 97 newborn pups from 10 pregnant dams were used for experiments to measure the amounts of milk intake as described below. The dam and her pups were kept in a single cage (26 cm × 42 cm × 18 cm) under standard laboratory conditions with a 12 h light/dark cycle and room temperature maintained at 22 ± 1 °C. Food and water were supplied ad libitum. The pups were housed with their dams for 3 weeks until P21 when they were weaned.

2.3. Survival rates and postnatal growth

After surgical procedures, the operated pups ($n = 11$) rarely died within 24 h. They included bilateral XII-trunk ($n = 4$), XII-med ($n = 3$), XII-lat ($n = 3$), and VII ($n = 1$) nerve-resected pups. As normal P1 pups could survive for at least 24 h without the dam, we excluded such cases from the experimental data for the survival rates regarded as effects of surgical damage. Thus, the survival rates were calculated as percentages of the numbers of surviving pups during the postnatal 3 weeks until P21 among the total number of the experimental animals, depending on each experimental group.

After nerve resection, pups were weighed every other day until P21. The mean values of the body weight were calculated for normal, control, and nerve-injured groups. Data are expressed as means ± S.D. Statistical significance of the means was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett test as post hoc analysis. P values less than 0.05 were considered significant.

2.4. Measurements of milk intake

P1 pups were subjected to nerve resection as described above. After surgery, the pups were housed with the dam. Their suckling capabilities were estimated on P4, P7, and P14 in the following manner. Prior to lactation, the pups were removed from the dam's cage and kept apart from the dam in a box (P4, P7: 12 cm × 13 cm × 9 cm; P14: 13 cm × 25 cm × 9 cm) made of thick paper for 4 h. The temperature in the box was kept at 27 °C and the humidity was kept at 30 ± 2%. Just prior to lactation, the pups were induced to excrete urine adequately by pressing the abdomen and stimulating the external urethral orifice and anus with tissue paper, and then their body weight was measured. Macroscopic views of the pups' abdomens just before lactation showed that the pups did not contain milk in the stomachs. The pups were placed back in a dam's cage and she was allowed to lactate for 1 h. Macroscopic views of the pups' abdomens just after lactation showed that the pups with milk intake contained milk in the stomachs and the pups with no milk intake did not contain milk in the stomachs. After 1-h lactation, the pups' body weight was re-measured. The milk intake of each individual pup was determined as the difference in body weight just before and after lactation. In the XII nerve-injured pups, body weight loss sometimes occurred after lactation due to lack of milk intake and transepidermal water loss (Kahn et al., 1987; Thijs et al., 1996). In such cases, no milk was detected in the stomach of the pups after lactation, and thus we regarded their milk intake as zero. The measurements were performed twice a day, and each pup's milk intake was defined as the average value. The mean values of milk intake were calculated for normal, control, and nerve-injured groups. Data are expressed as means ± S.E.M. Statistical significance of the means was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett test as post hoc analysis. P values less than 0.05 were considered significant.

3. Results

3.1. Survival rates

At the early neonatal stage (P1), the XII and VII nerves were transparent and unmyelinated. All the bilateral XII-trunk, XII-med, and XII-lat nerve-resected pups showed a continual decrease in body weight, and died between P3 and P5. In the unilateral XII nerve-resected pups, survival rates were quite different according to the nerve resected. The unilateral XII-trunk and XII-med nerve-resected pups were divided into two groups with a continual decrease in body weight and an initial decrease followed by an increase in body weight. The former group died between P3 and P5, while the latter group survived. Survival rates of the unilateral XII-trunk and XII-med nerve-injured pups were 38% (23/61) and 24% (16/68), respectively. With the exception of the rare occurrence of non-surviving cases (8%: 3/39), the XII-lat nerve-resected pups did not show a decrease in body weight even just after nerve resection and showed a high survival rate (92%: 36/39). All the unilateral and bilateral VII nerve-resected pups showed a continual increase