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Orosensory deprivation alters taste-elicited c-Fos expression in the parabrachial nucleus of neonatal rats

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ABSTRACT

In the present study we examined the effects of neonatal orosensory deprivation on taste-elicited gustatory activity in the rat parabrachial nucleus (PBN) using the functional anatomical marker c-Fos. Animals in three groups (GG, GO and GM) received gastric cannula implantation surgery on postnatal day 9 (P9). Animals in the fourth group (MR) did not receive any surgery. GG rats were fed by infusion of artificial milk directly into the stomach. GO rats were fed by intraoral infusion of artificial milk. GM and MR rats were reared by their mother with free access to mother's milk, water and rat chow. Rats from all groups were similar in body weight and length by P21. On P21 rats in all groups were intraorally presented with 0.5 M sucrose solution and the brains were extracted and processed for c-Fos immunohistochemistry. Taste-elicited c-Fos expression in both the gustatory waist area, and the external lateral subnucleus of the PBN in rats in the GG group was significantly more robust than in the other three groups. These findings suggest a substantial alteration in orosensory-evoked neuronal response in this nucleus, due to sensory or motor deprivation during a critical developmental stage.

1. Introduction

The development of normal feeding in human infants is dependent on experience with sensory reception of oral stimuli (Ayano et al., 2000). Often, infants either born prematurely or possessing congenital diseases need temporary nutritional support via tube feeding. Frequently, these infants display feeding impairments and motor speech delays (Bier et al., 1993; Jennische and Sedin, 1998, 1999; Hawdon et al., 2000; Dodrill et al., 2004). It is possible in these cases that impaired oral feeding is due to hypersensitivity in the oropharyngeal region. Weaning is a critical period for the development of normal feeding (Illingworth and Lister, 1964), and it is likely that orosensory experience during this period is crucial. However, there exist few basic studies investigating the significance of sensory reception in the development of feeding behavior.

Mammals possess a functional sense of taste at birth, although the gustatory system continues to develop into adulthood (for reviews see Mistretta, 1991; Barlow, 2000; Mistretta and Hill, 2003; Krimm, 2007). There is copious evidence that development in the taste central nervous system (CNS) of rodents may be altered prenatally by environmental manipulation, such as low-sodium diet fed to the dam (Krimm and Hill, 1997; Mangold and Hill, 2007). Studies also show that central gustatory development can be altered by manipulation of feeding in neonatal animals. For example, Lasiter and Diaz (1992) demonstrated that rats receiving artificial rearing (AR) via an intragastric cannula from postnatal days 4–14 (P4–P14) possess reduced terminal field volumes in the nucleus of the solitary tract (NST) as compared with mother-reared controls (Lasiter and Diaz, 1992). Further studies using AR rats demonstrated that this altered neuroanatomical development was specifically due to lack of gustatory stimulation (Lasiter, 1995). On the other hand, taste exposure during the suckling period was found to be unnecessary for the development of normal taste behavioral responses in rats (Bernstein et al., 1986).

The purpose of the current study was to examine how differences in feeding state during the critical weaning period affect gustatory-evoked neural activity in the taste CNS of AR rats. This was accomplished by comparing taste responses in rats fed during the critical weaning period (P9–P20) via either a gastric fistula or an intraoral cannula. Once the rats reached P21, taste-evoked activity was measured in the parabrachial nucleus (PBN). Studies examining c-Fos immunoreactivity (Fos-IR) in this region following intraoral stimulation with various tastants suggest the existence of a "taste map" in the PBN (Yamamoto et al., 1994; Yamamoto, 2006).

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Distinct patterns of Fos-IR across PBN subnuclei are evoked by stimuli classified as hedonically positive, such as sweeteners; a different pattern is evoked by aversive stimuli such as quinine. We hypothesized that rats fed intragastrically, without oral stimulation, would possess diminished or altered Fos-IR in the PBN in response to sweet taste stimulation. Sweet-tasting stimuli evoke appetitive or consumatory behavior in rats, and evoke responses distinct groups of gustatory neurons in the CNS (for review see Yamamoto, 2003). Sweeteners also elicit including suckling, a consumatory behavior, in neonatal rats (Kozlov et al., 2006).

2. Methods

The study was approved by the Animal Care and Use Committee Standards of Showa University (authorization no. 19006), and animals were treated in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised 1996.

2.1. Subjects

A total of 28 male Sprague–Dawley neonate rats (Sankyo Laboratory, Tokyo, Japan), weighing between 5.0 and 7.0 g at the start of the experiments were used. 28 of these were reared with their mother in a colony room for 8 days after birth (P1–P8), and then randomly assigned to one of four experimental groups (see below). These were reared with their mother until P21. All rats were housed in an animal colony room with a 12 h light/12 h dark cycle (lights on at 0800 h, off at 2000 h) and constant temperature ($25 \pm 1\,^{\circ}C$) and humidity (50–70%). Seven additional rats were excluded from the final number due to decannulation, leakage of artificial milk from the stomach, or excretion problems during the course of the experiments.

2.2. Surgery and groups

2.2.1. Gastrostomy surgery

The animals were arbitrarily divided into the GG (gastrostomy + intragastric infusion), GO (gastrostomy + intraoral infusion), GM (gastrostomy + mother rearing) and MR (mother rearing) groups consisting of seven rats each (Fig. 1). On day 9 (P9) rats in the GG, GO and GM groups were anesthetized with diethyl ether and polyethylene tubing gastric cannula (PE10, Becton Dickinson and Co., NJ, USA) was implanted in the fore-stomach as described previously (Ooka et al., 2008). After surgery, the GG and GO animals were returned to the colony and individually housed in plastic home cages to prevent decannulation. Animals in the GG group were reared by intragastric infusion of sterilized artificial milk (Bean Stalk Snow Co., Ltd., Tokyo, Japan). The milk was first infused into the stomach 6–12 h after surgery. The total volume of artificial milk infused on P9 was 2–3 ml. From P10–21, the volume of artificial milk for 1 day was calculated by the following formula: postnatal day $\times$ 0.65 ml (modified from Messer et al., 1969). Volume for one infusion was 1–2 ml. Infusion started at 0800 h and was given every 2 h at a rate of 0.5 ml/min. Animals in the GO group were fed in a similar fashion as those in the GG group except that they were infused intraorally with artificial milk. Both groups were maintained under this feeding schedule until P21. After surgery, the GM rats were returned to their mother and allowed free access to dam milk, water and rat chow (Nosan Co., Kanagawa, Japan) until P21. Animals in the MR group did not receive gastrostomy surgery, and were reared by their mother with free access to dam milk, water and rat chow throughout the experiments.

2.2.2. Intraoral cannula implantation

We developed an original method for intraoral cannula implantation due to the fact that the conventional method, where the cannula is anchored to the skull with screws (e.g. Yamamoto and

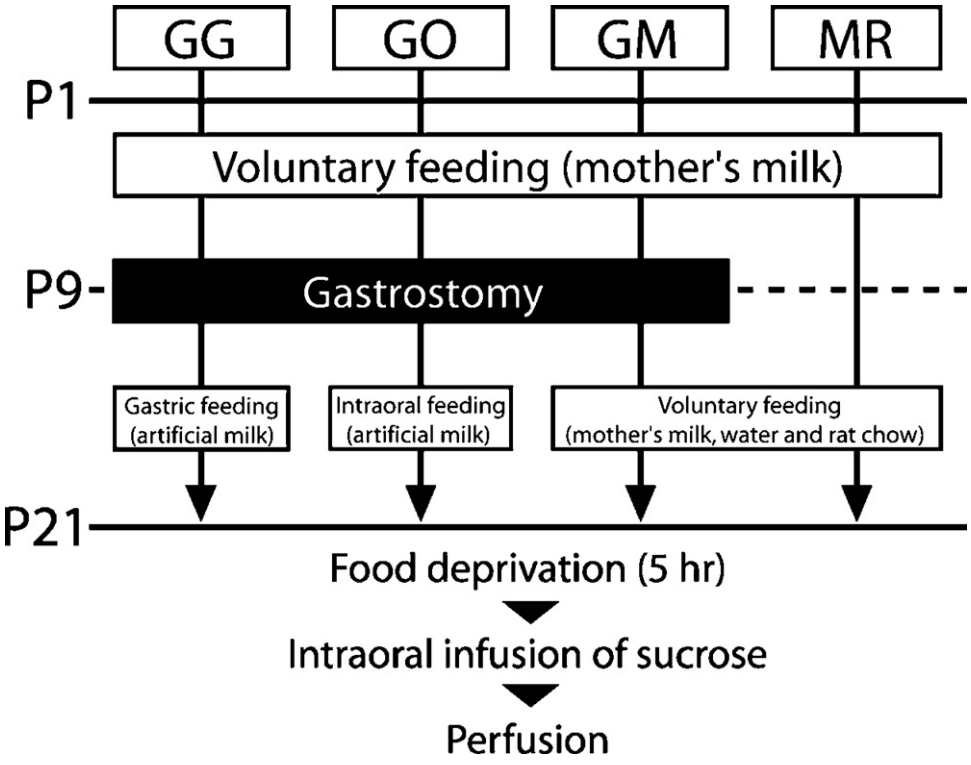

Fig. 1. Schematic drawing of the procedures and timelines for each of the four experimental groups. GG, gastrostomy + intragastric infusion; GO, gastrostomy + intraoral infusion; GM, gastrostomy + mother rearing; MR, mother rearing.