

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Fos expression induced by milk ingestion in the caudal brainstem of neonatal rats**Teresa Morales^{a,*}, Leticia Aguilar^a, Eugenia Ramos^a, Flavio Mena^a, Cernel Morgan^b^aDepartamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, 76230, Querétaro, Mexico^bDepartment of Nutrition and Food Science, Texas A&M University, College Station, TX 77843-2253, USA

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ABSTRACT

Prominent Fos expression in the nucleus of the solitary tract (NTS) related to feeding has been reported in the brainstem of adult animals. In this study, we used a Fos-guided immunohistochemical approach to determine the brainstem areas activated specifically in response to milk ingestion in rat pups at two different ages. Rats at 9 or 18 days postpartum were isolated from the mother for a 6-h period, after which they were returned to the mother for a suckling period of either 5 or 90 min and then perfused at 90 min after the beginning of suckling. Control groups were sacrificed before or after the 6-h-deprivation period and showed little or no Fos-ir. In contrast, a 90-min-suckling episode after 6 h of deprivation induced strong Fos-ir in the caudal regions of the NTS and in the spinal nucleus of the trigeminal (SPV). Moderate expression was observed in the rostral NTS and in the nucleus raphé obscurus. In rat pups that suckled for only 5 min, the main area activated was the SPV. Fos immunostaining was detected in only 1% of the catecholaminergic neurons from the NTS after milk ingestion. The experimental design employed here allowed us to distinguish brainstem areas activated by milk ingestion from those activated by suckling action in rat pups. In contrast to adult rats, catecholaminergic neurons from the caudal NTS seem to contribute little to the regulation of feeding at this age.

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1. Introduction

In the newborn rat, ingestion is stimulated by dehydration and inhibited by gastric distension (Hall, 1985). In adult animals, Fos immunodetection has identified CNS areas that are activated by feeding and/or distension of the stomach. Studies in the brainstem of adult animals related to feeding and stomach distension have shown prominent Fos expression in the areas of the nucleus of the vagal complex, such as the nucleus of the solitary tract (NTS), area postrema, and dorsal motor nucleus of the vagus (Fraser and Davison, 1993; Fraser

et al., 1995; Rinaman et al., 1998; Willing and Berthoud, 1997; Berthoud, 2002). Fos has also been observed in neurons from the medullary reticular formation mostly related to stimulation of the oromotor circuitry (DiNardo and Travers, 1997).

Although feeding-induced Fos expression has been investigated in adult animals, fewer studies have focused on feeding-induced activation of brainstem neurons in immature animals during the lactation phase. Recent reports have shown that feeding-induced Fos expression in the NTS of neonate rats is similar to that reported in adults after ingestion of a satiating meal (Hironaka et al., 2000), as well as the Fos activational

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response in NTS to CCK or gastric preloads (Blumberg et al., 2006, 2007). Also, oxytocin is elevated in the plasma of 10-day-old rats after induction of gastric distension with either mother's milk or saline, indicating that the vagal-hypothalamic axis becomes mature between 2 and 10 days of age (Nelson et al., 1998).

Early postnatal life represents a unique opportunity to examine the neural basis of the ingestive system (Rinaman, 2006) and research in the field of ingestion at early postnatal ages is required to better understand the maturation of the circuitries regulating this function. In this study we have used a fos-guided immunohistochemical approach to identify brainstem areas responding specifically to stomach filling as compared to areas activated solely by the action of suckling in rat pups at two different ages, 9 and 18 days of age. The experimental model employed here has advantages over models employed previously by others (Hironaka et al., 2000), because in our model we are detecting Fos activation response induced by either the action of suckling or the complete set of stimuli that involve nursing and milk ingestion. Also, our model

avoids behavioral-conditioning mechanisms (Angeles-Castellanos et al., 2005) involved in the nursing episode.

The second purpose of the present study is to determine the extent to which the catecholaminergic neurons, located in the caudal brainstem (A2 and A1 areas) that are known to project to the hypothalamus and respond to food intake in adults, respond to milk ingestion at these ages. It has been reported that 10 to 40% of catecholaminergic neurons in these areas in adult rats show an activation response after gastric filling or distension (Willing and Berthoud, 1997; Rinaman et al., 1998).

2. Results

2.1. Fos expression in the caudal brainstem after milk ingestion

Analysis of coronal sections of the caudal brainstem from rat pups subjected to our experimental conditions proved that the

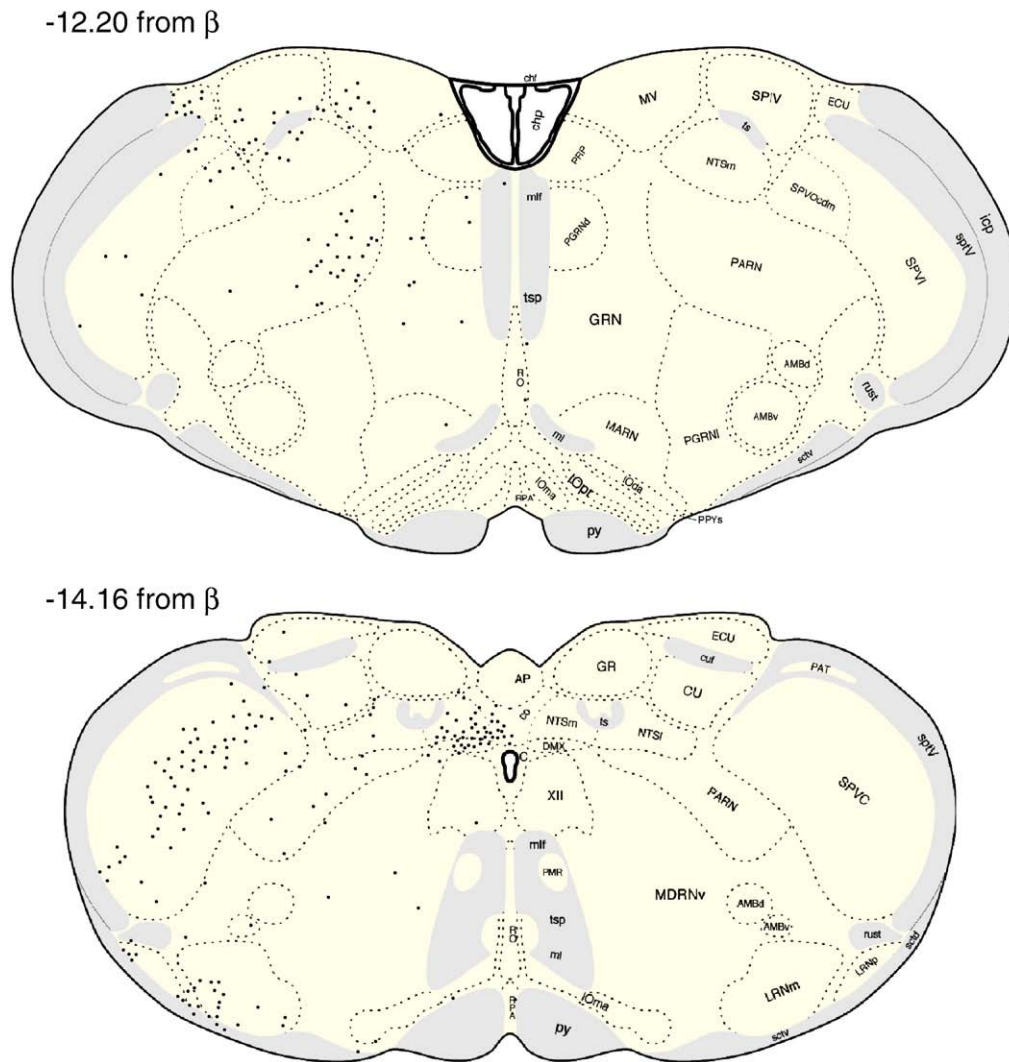


Fig. 1 – Diagram of two different levels (rostral and caudal) of NTS showing the Fos expression induced by milk ingestion in 9-day-old rat pups that suckled for 90 min after 6-h-deprivation period. Fos expression was moderate in the rostral gustatory part of the NTS, but stronger in the caudal NTS and SPV in pups that suckled for 90 min after 6 h of deprivation. Abbrev. NTS: nucleus of solitary tract; ts: solitary tract; SPV: spinal nucleus of the trigeminal.

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