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Research Report

Patterns of fos activation in rat raphe nuclei during feeding behavior

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ABSTRACT

To analyze the differential recruitment of the raphe nuclei during different phases of feeding behavior, rats were subjected to a food restriction schedule (food for 2 h/day, during 15 days). The animals were submitted to different feeding conditions, constituting the experimental groups: search for food (MFS), food ingestion (MFI), satiety (MFSa) and food restriction control (MFC). A baseline condition (BC) group was included as further control. The MFI and MFC groups, which presented greater autonomic and somatic activation, had more FOS-immunoreactive (FOS-IR) neurons. The MFI group presented more labeled cells in the linear (LRN) and dorsal (DRN) nuclei; the MFC group showed more labeling in the median (MRN), pontine (PRN), magnus (NRM) and obscurus (NRO) nuclei; and the MFSa group had more labeled cells in the pallidus (NRP). The BC exhibited the lowest number of reactive cells. The PRN presented the highest percentage of activation in the raphe while the DRN the lowest. Additional experiments revealed few double-labeled (FOS-IR+5-HT-IR) cells within the raphe nuclei in the MFI group, suggesting little serotonergic activation in the raphe during food ingestion. These findings suggest a differential recruitment of raphe nuclei during various phases of feeding behavior. Such findings may reflect changes in behavioral state (e.g., food-induced arousal versus sleep) that lead to greater motor activation, and consequently increased FOS expression. While these data are consistent with the idea that the raphe system acts as gain setter for autonomic and somatic activities, the functional complexity of the raphe is not completely understood.

1. Introduction

The raphe nuclei, considered to be the main source of encephalic serotonin, constitute a non-homogeneous neuronal population located in the midline of the brainstem, exhibiting diverse morphological, chemical and functional characteristics. Some of their known functions comprise different phases of feeding behavior, which can be separated into motivational, endocrine, and motor somatic and autonomic components. Such components include increased endocrine and exocrine pancreatic secretion (Krowicki and Hornby, 1995; Park et al., 1995; Yang et al., 2002), as well as somatic and visceral alterations in feeding behavior (Leibovits and Stanley, 1986), increased gastric secretion (Yang et al., 1990; 1993), gastric motility (Krowicki and Hornby,

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Table 1 – Experimental conditions during food restriction schedule, duration of meal feeding, and control groups

	Group	Experimental phase	Stimulus duration	Time of perfusion
Food restriction	MFC	Restriction control	0 min	07:30 h
	MFS	Search	30 min	10:00 h
	MFI	Ingestion	30 min	10:00 h
	MFSa	Satiety	120 min	11:30 h
Control	BC	Baseline conditions	Ad libitum	11:00 h

1994), respiratory (Richerson et al., 2001, Wang et al., 2001; Bradley et al., 2002) and circadian cycle regulation (Shen and Semba, 1993; Vertes and Kocsis, 1994; Glass et al., 2000).

Serotonin appears to participate in the control of feeding behavior (Blundell, 1977; Simansky, 1996; Kaye et al., 1998; Schuhler et al., 2005) and maybe involved in the modulation of hunger and satiety (Blundell and Hill, 1987), depending on the receptor subtype involved (Bovetto and Richard, 1995; Lin and York, 2005; Somerville et al., 2007). Serotonergic transmission from the dorsal (DRN) (Fletcher and Coscina, 1993; Ohliger-Frerking et al., 2002) and median (MRN) (Wirtshafter, 2001) raphe nuclei to the ventromedial hypothalamus contributes to feeding regulation. Serotonergic neurons also exert facilitatory effect on the trigeminal motoneurons (Ribeiro-do-Valle, 1997; Ribeiro-do-Valle and Lucena, 2001).

Several studies have employed c-fos induction (an immediate early gene) as a marker of neuronal activation by various feeding stimuli (Olson et al., 1993; Emond and Weingarten, 1995; Fraser et al., 1995). FOS expression is greatly reduced or absent under normal basal conditions, but is quickly induced in the neuronal nucleus in response to effective stimuli, reaching peak levels within 90 to 120 min (Herdegen and Leah, 1998; Hoffman and Lyo, 2002). Since FOS is a nuclear protein, its expression can be combined with other immunohistochemical techniques to demonstrate co-labeling with a cytoplasmatic antigen, such as a neurotransmitter (e.g., serotonin) or an enzyme (e.g., tryptophan hydroxylase).

The present study explores the hypothesis that there may exist a differential activation of neurons in the various raphe nuclei during different phases of feeding behavior. To this end, we systemically examined FOS expression in the various raphe nuclei (as a cellular marker of neuronal activation) in rats submitted to a food-entrainment schedule.

2. Results

FOS protein expression was analyzed through the raphe system during different phases of feeding behavior. Table 1 (see Experimental procedures section) summarizes the different experimental groups, divided according to the feeding stimulus presented to the animals: food restriction control (MFC), search for food (MFS), ingestion of food (MFI) and satiety (MFSa). A baseline condition (BC) group was also analyzed as further control.

Table 2 shows the number of FOS-immunoreactive (FOS-IR) neurons for each experimental group in the rostral and caudal raphe nuclei. The rostral raphe comprises the linear (LRN), dorsal (DRN), median (MRN) and pontine (PRN) nuclei; while the caudal raphe comprises the magnus (NRM), pallidus (NRP) and obscurus (NRO) nuclei.

The overall analysis clearly revealed that the approach used was effective in inducing at least 5 times more FOS-IR neurons in all groups compared to the baseline group, except for the NRO in which it was between 2 and 3 times higher. Interestingly, the MFC group (food restriction control) presented the highest number of FOS-IR neurons. This is consistent with food anticipatory activity being an adaptative mechanism to optimize food consumption in food-entrained animals (Marques and Menna-Barreto, 1997; Takase et al., 2000).

The two-way repeated-measures ANOVA (treatment x nuclei) and the Bonferroni's multiple comparison test revealed statistical differences in the number of FOS-IR cells in the raphe nuclei among the experimental groups. These differences in FOS expression were most evident in the rostral raphe. In the LRN, only the MFI group was statistically different from the baseline group. The rank order (highest to lowest) of the number of FOS-IR neurons seen in the DRN across the different experimental conditions was: MFI>MFC>MFS>MFSa (see Table 2 and Fig. 1). In addition, in the DRN, the MFI was statistically different from the MFS and MFSa. The MFS and MFI groups were considered the most relevant groups in this study because they were perfectly balanced: both were submitted to 30 min of feeding stimuli (search for food or ingestion of food) and perfused at the same time period (10:00 h).

Among the caudal raphe nuclei (NRM, NRP and NRO), only the NRP showed significant differences in the number of FOS-IR neurons between the MFS and MFSa groups. Although no

Table 2 – Number of FOS-IR neurons for each experimental group in the rostral and caudal raphe nuclei

		Baseline condition (BC)	Restriction control (MFC)	Search (MFS)	Ingestion (MFI)	Satiety (MFSa)
Rostral nuclei	LRN	58±15	535±110	262±59	620±90*	470±44
	DRN	134±14	1572±39*	1228±97*	1744±223*,§	1040±94*,†
	MRN	68±13	783±91*	535±47	573±50*	390±32
	PRN	58±8	480±31	345±32	316±17	265±18
Caudal nuclei	NRM	102±11	553±40	352±74	518±23	546±58
	NRP	190±39	682±149	685±80*	587±74	747±118*
	NRO	149±24	418±105	169±45	275±66	259±44

Values are means±SEM; n=5/group.
*$p<0.05$ vs BC; §$p<0.05$ vs MFS; †$p<0.05$ vs MFI; by ANOVA and Bonferroni's multiple comparison test. For purposes of comparing the data, the two most relevant groups are highlighted.