available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

The dynamics of neuronal activation during food anticipation and feeding in the brain of food-entrained rats

*Anne-Marie Poulin, Elena Timofeeva**

Centre de recherche de l'Hôpital Laval, Pavillon Marguerite d'Youville Y3150, 2725 Chemin Sainte-Foy, Québec (QC), Canada G1V 4G5

ARTICLE INFO

Article history:
Accepted 15 June 2008
Available online 21 June 2008

Keywords:
Food anticipation
Restricted feeding schedule
Food-entrainable oscillator
c-*fos*
Neuronal activation
CRF

ABSTRACT

Rats can anticipate a daily mealtime when they are maintained on restricted feeding schedules (RFS). Neural substrates of the food-entrainable oscillator (FEO) are not yet fully understood. The numerous lesions of a single brain region failed to abolish the behavioral anticipation of a daily meal, suggesting that the FEO may be represented by a distributed neuronal network. The present study was designed to detect the dynamics of neuronal activation, using as a marker the expression of c-*fos* mRNA in the brain of rats subjected to 2-hour daily RFS, 3, 2 and 1 h before the expected meal, at the time of the usual feeding, and 1 h after feeding. We also aimed to clarify whether the increase in plasma corticosterone in food-anticipating rats coincides with the increase in expression of corticotropin releasing factor (CRF) mRNA in the paraventricular hypothalamic nucleus (PVH). The obtained results revealed that the neuronal activation occurring 3 h before the expected meal was not confined to one brain structure, but was evident in the anterior hippocampal continuation and septohippocampal nucleus (AH/SHi), the anterior part of the paraventricular thalamic nucleus (PVTa), and the dorsomedial hypothalamic nucleus (DMH), thus representing distributed septohippocampal-thalamo-hypothalamic circuitry that may act as the FEO. The pattern of neuronal activation after feeding was different from that detected during food anticipation for some specific nucleus or subregions. The increase in plasma corticosterone during food anticipation was not accompanied by an increase in CRF mRNA levels, suggesting that factors other than CRF are involved in the control of adrenocortical secretion under RFS.

* *Corresponding author*. Fax: +1 418 656 4942.
E-mail address: Elena.Timofeeva@ap.ulaval.ca (E. Timofeeva).

Abbreviations: ACh, acetylcholine; ACTH, adrenocorticotropic hormone; AD, anterodorsal thalamic nucleus; AH/SHi, anterior hippocampal continuation and septohippocampal nucleus; AHA, anterior hypothalamic area; BLA, basolateral amygdala; CeA, central nucleus of amygdala; CRF, corticotropin releasing factor; DLG, dorsal lateral geniculate nucleus; DMH, dorsomedial hypothalamic nucleus; DMHc, dorsomedial hypothalamic nucleus, compact part; DMHv, dorsomedial hypothalamic nucleus, ventral part; DTg, dorsal tegmental nucleus; FAA, food-anticipatory activity; FEO, food-entrainable oscillator; HPA, hypothalamic–pituitary adrenal axis; LEO, light-entrainable oscillator; LHA, lateral hypothalamic area; LM, lateral mammillary nucleus; NTS, nucleus of the solitary tract; PB, parabrachial nuclei; PVH, paraventricular hypothalamic nucleus; PVHam, paraventricular hypothalamic nucleus, anterior magnocellular part; PVHmm, paraventricular hypothalamic nucleus, medial magnocellular part; PVHp, paraventricular hypothalamic nucleus, parvocellular part; PVT, paraventricular thalamic nucleus; PVTa, paraventricular thalamic nucleus, anterior part; PVTp, paraventricular thalamic nucleus, posterior part; RFS, restricted feeding schedules; SCN, suprachiasmatic nucleus; SG, suprageniculate thalamic nucleus; SON, supraoptic nucleus; SuG, superficial gray layer of the superior colliculus; SuM, supramammillary nucleus; VMH, ventromedial hypothalamic nucleus

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2008.06.039

1. Introduction

The appropriate feeding behavior is essential for the survival of animals. This behavior should be coordinated with environmental conditions, such as the light–dark cycle and food availability, and internal physiological requirements, such as maintaining gastrointestinal functions and energy balance. The suprachiasmatic nucleus (SCN), which is regarded as the light-entrainable oscillator (LEO), orchestrates multiple circadian rhythms in the organism that are regulated according to environmental light/dark signals conveyed by the retinohypothalamic tract directly to the SCN (Hastings et al., 2003; Lowrey and Takahashi, 2004). However, when food availability is restricted to a few hours scheduled at a fixed time of the day, animals develop food-entrained circadian rhythms. Rats maintained on restricted feeding schedules (RFS) exhibit the behavior called food-anticipatory activity (FAA) that is manifested within the hours preceding food access by arousal and increased exploration and foraging locomotion (Stephan, 2001). Core temperature, plasma corticosterone, and free fatty acids shift their phase in anticipation of a daily meal (Diaz-Munoz et al., 2000; Escobar et al., 1998; Krieger, 1979). Biological rhythms associated with RFS persist after complete bilateral lesions of the SCN (Marchant and Mistlberger, 1997; Mistlberger, 1993), indicating the presence of a food-entrainable oscillator (FEO) that may function independently of the SCN.

Although much effort has been invested in the elucidation of a FEO, the possible elements of this oscillator are not yet fully understood. The persistence of FAA after adrenalectomy (Boulos and Terman, 1980), subdiaphragmatic vagotomy (Comperatore and Stephan, 1990; Moreira and Krieger, 1982), and capsaicin-induced deafferentiation (Davidson and Stephan, 1998) argues against the peripheral location of the FEO. Moreover, the importance of the liver or the pancreas as a possible component of the FEO was disproved in studies involving cirrhotic (Escobar et al., 2002) and diabetic (Davidson et al., 2002) rats.

In an attempt to locate the FEO in the central nervous system, several groups have produced ablations of potential anatomic substrates of this oscillator in the brain and explored its effects on the expression of FAA. In food-entrained rats, bilateral lesions of the ventromedial hypothalamic nucleus (VMH) did not prevent FAA or the preceding corticosterone peak (Honma et al., 1987; Mistlberger and Rechtschaffen, 1984). Likewise, bilateral lesions of the lateral hypothalamic area (LHA) and the paraventricular hypothalamic nucleus (PVH) failed to abolish FAA (Mistlberger and Rusak, 1988), and similar negative results were obtained after the lesion of the olfactory bulb (Davidson et al., 2001b), components of the limbic system (Mistlberger and Mumby, 1992), and the area postrema (Davidson et al., 2001a). Recent claims that the dorsomedial hypothalamic nucleus (DMH) is critical for the development of FAA (Gooley et al., 2006) appear to be controversial, as other groups working with rats (Landry et al., 2006) and mice (Moriya et al., 2007) have been unable to confirm that DMH ablation eliminates food-anticipatory behavior.

Because the identification of a single structure as a substrate of FEO has not been successful, it has been suggested that the FEO may be represented by a distributed and possibly redundant neuronal network interacting with peripheral systems related to digestion and energy metabolism (Escobar et al., 1998; Stephan, 2001). This inference is supported by a significant attenuation of the expression of FAA after genetic ablation of the clock-related Per2 gene (Feillet et al., 2006) that is expressed in a number of brain structures, including the cortex, limbic system, thalamus, and hypothalamus (Shieh, 2003).

In the brain of rats subjected to RFS, the expression of the Fos protein, the marker of neuronal activation, was detected as early as 2 h before the expected meal in some limbic structures such as the lateral septum and nucleus accumbens, and in the DMH, LHA, and paraventricular thalamic nucleus (PVT) at the time of expected feeding (Angeles-Castellanos et al., 2004; Angeles-Castellanos et al., 2007; Johnstone et al., 2006; Mendoza et al., 2005; Nakahara et al., 2004). Expression of c-*fos* mRNA, which precedes synthesis of the Fos protein, has not been detected before in the brain of food-anticipating rats.

The present study investigated the dynamics of neuronal activation in the rat brain during food anticipation at 3, 2, and 1 h before the expected meal, at the time of usual access to food and following 1 h of feeding. This experimental design was aimed to reveal which brain structures were activated and how this activation spread during food anticipation, and compare the neuronal activation during food anticipation and feeding. We hypothesized that the key structures triggering central activation and behavioral response would activate early during the period of anticipation and that the pattern of neuronal activation during food anticipation would not be similar to that described for feeding (Timofeeva et al., 2002).

It is known that behavioral activation during food anticipation coincides with an increase in the plasma levels of corticosterone. On the other hand, there is no evidence that the levels of corticotropin releasing factor (CRF) transcript in the parvocellular part of the PVH or serum ACTH are altered during FAA (Belda et al., 2005; Pesonen et al., 1992). Our previous studies have demonstrated that an increase in plasma corticosterone during acute fasting was not accompanied by neuronal activation of the PVH neurons or by an increase in PVH CRF expression (Timofeeva and Richard, 1997, 2001; Timofeeva et al., 2002). The increase in fasting plasma corticosterone was attributed to a reduction in the plasma clearance rate of this hormone (Kiss et al., 1994; Woodward et al., 1991) aimed to mobilize the energy resources in this catabolic state (Richard et al., 2000). In the present study, we aimed to clarify whether the increase in plasma corticosterone in food-anticipating rats coincides with an increase in the expression of c-*fos* or CRF mRNAs in the PVH.

The results have revealed that in the brain of rats subjected to RFS, the septohippocampal, paraventricular thalamic, and dorsomedial hypothalamic nuclei were activated as early as 3 h before the expected meal. These brain regions may represent the food-entrainable brain oscillator and may trigger further central activation and behavioral food-anticipatory reaction. The pattern of neuronal activation during food anticipation was different from that induced by feeding for some specific brain regions or some subnuclei of the areas sensitive to both food anticipation and feeding conditions. Finally, the present data represent evidence that the anticipating increase in plasma corticosterone is not accompanied by an enhancement in c-*fos* and CRF PVH expression and