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A reassessment of the role of serotonergic system in the control of feeding behavior

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ABSTRACT

The role of serotonergic system in the feeding behavior was appraised by electrolytic lesions in the dorsal raphe nucleus (DRN) and administration of para-chlorophenylalanine (PCPA, 3 mg/5 μl, icv). Chronic evaluations were accomplished through 120 and 360 days in PCPA-injected and DRN-lesioned rats, respectively. Acute food intake was evaluated in fasted rats and submitted to injection of PCPA and hydroxytryptophan (LHTP, 30 mg/kg, ip). DRN-lesioned rats exhibited 22-80% increase in food intake up to sixth month, whereas the obesity was evident and sustained by whole period. In PCPA-injected rats was observed an initial increase in the food intake followed by hypophagy from 25th to 30th day and a transitory increase of body weight from 5th to 60th day. In the acute study, the LHTP reverted partially the PCPA-induced increase in food intake of fasted rats suggesting a sustained capacity of decarboxylation of precursor by serotonergic neurons. Slow restoration of the levels of food intake in DRN-lesioned rats reveals a neuroplasticity in the systems that regulate feeding behavior. A plateau on the body weight curve in lesioned rats possibly represents the establishment of a new and higher set point of energetic balance.

Key words: food ingestion, serotonergic system, dorsal raphe nucleus, electrolytic lesion, para-chlorophenylalanine, obesity.

INTRODUCTION

Evidences have implicated the serotonergic transmission in the feeding behavior. Basomedial nuclei of hypothalamus and lateral hypothalamic area, admittedly involved with feeding behavior control, are innervated by serotonin neurons from the midbrain raphe (Azmitia and Segal 1978, Parent et al. 1981). Studies with administration of serotonin precursors (Fernstrom and Wurtman 1971a, b, Fernstrom 1983), inhibitors of serotonin presynaptic uptake, serotonin releasers, inhibitor of tryptophan hydroxylase and receptor agonists of serotonin (Blundell 1984, 1991) are concordant with the serotonergic hypothesis of appetite modulation. Postsynaptic receptors 5HT1B and 5HT2C are the more relevant for the anorexigenic response in mammals and birds (Blundell 1984, 1991, Curzon 1990, 1991, Cedraz-Mercez et al. 2004, unpublished data). This statement was corroborated by Tecott et al. (1995), Nonogaki et al. (1998), Chou-Green et al. (2003) who showed that 5HT2C knockout mice develop hyperphagia and adiposity. In addition, hyperphagia and obesity after brain serotonin depletion induced by inhibition of tryptophan hydroxylase, or

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after neurotoxic lesion of serotonin neurons, have reinforced the serotonergic hypothesis (Breisch et al. 1976, Saller and Stricker 1976).

Recent studies demonstrated the participation of other two systems of food intake control, the leptin, hormone secreted by adipocytes after increase of fat in the adipose tissue and, the orexin A, a neuropeptide which is expressed in the lateral hypothalamus after fasting and subsequent energy depletion (Janeckova 2001, Rodgers et al. 2002). Leptin reduces food ingestion and additionally stimulates mechanisms within basomedial hypothalamus concerned to fat oxidation, energetic balance and, therefore, the body weight as well (Friedman and Halaas 1998, Grill and Kaplan 2002).

Subset of serotonin neurons within the raphe nuclei co-expresses serotonin transporter mRNA and leptin receptor mRNA (Finn et al. 2001). In this line of reasoning, Fernández-Galaz et al. (2002) evidenced the leptin uptake by serotonin neurons of the dorsal raphe nucleus (DRN) and Yamada et al. (2003) showed that the hypophagic effect of leptin is mediated by serotonergic activity and subsequent 5HT2C receptor stimulation. On the other hand, orexin A receptors were identified in serotonin neurons of the DRN on which its excitation possibly constitute a negative feedback loop for acute control of food ingestion, particularly carbohydrates (Brown et al. 2001).

However, evidences concerning the role of DRN ascending pathways in the regulation of feeding behavior are inconclusive. Observations concerning lesions are controversial in spite the above mentioned reports. Thus, Geyer et al. (1976) showed that electrolytic lesions of B7 area, corresponding DRN, didn't produce significant alterations in the food ingestion and gain of body weight, as well. When lesion was directed to B8 area, corresponding median raphe nucleus (MRN) the authors reported an increase of the food ingestion and body weight (Geyer et al. 1976, Blundell 1984). It is interesting to record that, these observations were made in 6 months old rats through 4 weeks. In opposing, Heym and Gladfelter (1982) don't evidenced increase of food ingestion in young rats in same range of age and body weight between 150 and 200 g. In addition to those contradictory data, no evidence has been reported regarding body weight gain time course relatively to food ingestion in a longer observation period.

In the current study we reassessed the role of the serotonergic system in the control of food ingestion. New approaches were now justified considering the convergence of recent evidences for the role of serotonergic circuitry of DRN in the regulation of appetite and satiety mechanisms. Acute experiments were carried out in adult rats which received para-chlorophenylalanine (PCPA) into brain lateral ventricle and were submitted to fasting on the 4^{th} day after microinjection and then treated with l-hydroxytryptophan (LHTP). In chronic experiments, adult rats were treated with PCPA or submitted to DRN lesion and evaluated by 120 and 360 days, respectively.

MATERIALS AND METHODS

Animals and General Procedures

Wistar male rats from Fundação Oswaldo Cruz weighing 270-280 g were employed after previous adaptation to metabolic cage during one week. The animals were maintained under *ad libitum* offer of food and water. Experimental protocols were performed in laboratory with temperature control (25°C) and lights on from 7:00 h to 19:00 h. Food ingestion was determined in the metabolic cages provided with chow container. Measurement of food ingestion was made by electronic precision scale in a cumulatively way, in the acute experiments and by 24-h interval in chronic evaluations. Experimental procedures were accomplished according to Brazilian College of Animal Experimentation and pertinent to Brazilian legislation.

Implantation of Canulae into Lateral Brain Ventricle and Microinjections

Intracerebroventricular (icv) microinjections were made by steel cannulae implanted bilaterally into

brain lateral ventricles in anesthetized rats (2.5% tri-bromoethanol, ip). Cannulae were placed by employing a stereotaxic device using the following coordinates: anterior-posterior, 0.9 mm posterior to bregma; lateral, 1.2-1.4 mm; vertical, 3.2-3.4 mm from skull calvaria (Paxinos and Watson 1986). Microinjections were made with a 10–μl Hamilton microsyringe.

Brain Serotonin Depletion

Brain serotonin depletion was produced by icv microinjection of para-chlorophenylalanine methyl ester (PCPA, Sigma, St Louis, Mo, USA) an irreversible inhibitor of the tryptophan hydroxylase (TPO) at the dose of 3 mg/5μl, bilaterally, during 2 minutes under light anesthesia as described elsewhere (Breisch et al. 1976, Koe and Weissman 1966, Reis et al. 1994, Cooper et al. 1996). Control group was treated with isotonic saline (5μl, icv, bilaterally). Acute experiments were fulfilled 4 days after icv microinjection. Chronic experiments were initiated after restoration of anesthesia effect.

Electrolytic Lesions of DRN

Electrolytic lesions of DRN were produced in rats anesthetized with tribromethanol (2.5%, ip), which were fixed in a Kopf stereotaxic device. DRN was placed through following coordinates (according to Paxinos and Watson atlas): anterior-posterior, 7.6–7.8 mm posterior to bregma; lateral, 0.0 mm; vertical, 6.2–6.4 mm from skull calvaria (Paxinos and Watson 1986). Lesions were produced by passing an anodal current (2 mA, DC, for 10 sec) through nickel-chrome electrode guided into DRN. Control group received identical maneuver except current delivery (sham lesion). Isolated group of 12 rats were sacrificed 30 days after DRN electrolytic lesion under profound anesthesia. Transcardiac infusion was made with saline and 10% formaldehyde and the brains were arrested for histological analysis. Confirmation of lesions was made by histological examination of coronal sections through the midbrain (10μm thickness) stained by cresyl violet.

Experimental Procedures

Investigations were carried out in three experimental sets:

1) PCPA-treated rats were 24 hours fasted from the 4th day post-microinjection (N = 10). In following day, fasted rats were given l-hydroxytryptophan, immediate precursor of serotonin synthesis (LHTP, Sigma, St Louis, Mo, USA) at the dose of 30 mg/kg, ip. Control group received isotonic saline (1ml/kg, ip) (N = 12). Both groups were further returned to metabolic cages. Food ingested was determined cumulatively through 3 hours from 19:00 hours.

2) PCPA-treated rats recovered from anesthesia effect were returned to cages (N = 10) where chronic evaluation of food ingestion and body weight were made during 120 days. Control group was injected with saline (N = 12) and also transferred to cages.

3) DRN-lesioned (N = 10) and sham lesion rats were returned to cages after recovery of anesthesia effect and observed through 360 days in which food ingested and body weight were determined.

Statistical analysis

Results were reported as means $\pm$ SE. Data were analyzed statistically by two-way analysis of variance with repeated measures, and the significance between means was determined by the Newman-Keuls test. Differences between means were considered to be significant when $P < 0.05$.

RESULTS

PCPA-treated and -fasted rats displayed more intense food ingestion compared to controls (Figure 1). Previous administration of LHTP reduced significantly the ingestive response but at superior level in comparison to controls ($P < 0.05$, at 60, 120 and 180 minutes, respectively) (Figure 1). In chronic evaluation, PCPA-treated and control rats showed a transitory phase of hypophagy resulting of surgery

until the second day after central administration. However, from the 5th day serotonin-depleted rats presented an intense hyperphagia with gradual decrease from 15th day ($P < 0.05$) (Figure 2). After transitory phase of hypophagy between 25th and 30th day PCPA-treated rats returned to control levels of food ingestion. At 40th day the mean values of food ingestion in PCPA-treated group were equivalent to controls. Body weight gain initiated on 5th day and maintained high in plateau way up to 60th day ($P < 0.05$) and became comparable to controls at 90th day ($P > 0.05$) post-injection (Figure 3).

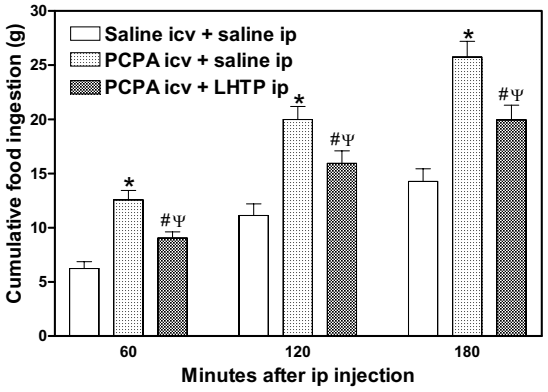

Fig. 1 – Acute effect of the previous icv PCPA microinjection *vs* ip LHTP injection on the food intake in nocturnal fasted rats. Data are presented as mean ± standard error at 60, 120 and 180 min. $^{*\Psi}P < 0.05$ compared to control group. $^{\#}P < 0.05$ compared to PCPA, icv + saline, ip (ANOVA and Newman-Keuls test).

Typical electrolytic lesions of the DRN extended in anterior-posterior direction (AP, 7.2-8.3 mm posterior to bregma) along rostral-dorsal and ventral-medial regions (Figure 4). DRN-lesioned rats displayed an intense hyperphagia from the 5th day post-surgery ($P < 0.05$) (Figure 5). This response maintained by 145 days on which the level of food ingestion remained higher than 30 g, whereas the sham lesioned rats continued in the 20 g level. From the 150th to 360th day the levels of food ingested in DRN-lesioned group were comparable to controls. Body weight of the DRN-lesioned rats increased drastically from the 5th day in comparison to controls ($P < 0.05$) (Figure 6). This feature maintained until the moment from which it developed a plateau significantly higher than controls ones, in spite the levels of food ingested between groups have been equivalent ($P < 0.05$) (Figure 6).

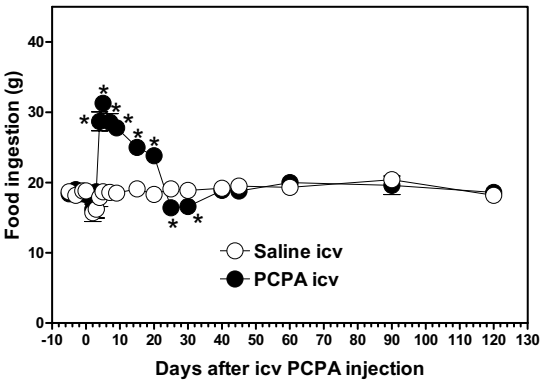

Fig. 2 – Chronic effect of the icv injection of PCPA on food intake in rats. Data are presented as mean ± standard error. $^{*}P < 0.05$ compared to sham lesion group (ANOVA and Newman-Keuls test).

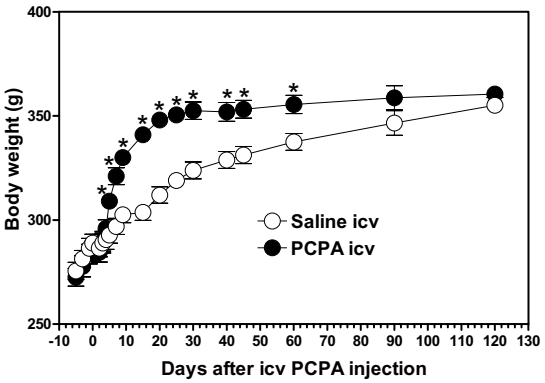

Fig. 3 – Chronic effect of the icv injection of PCPA on the body weight in rats. Data are presented as mean ± standard error. $^{*}P < 0.05$ compared to sham lesion group (ANOVA and Newman-Keuls test).

DISCUSSION

Results showed that PCPA-treated fasted rats expressed more intense ingestive response than the controls, presumably because the brain serotonin depletion exacerbated the orexigenic pathways activity. LHTP administration reverted partially the

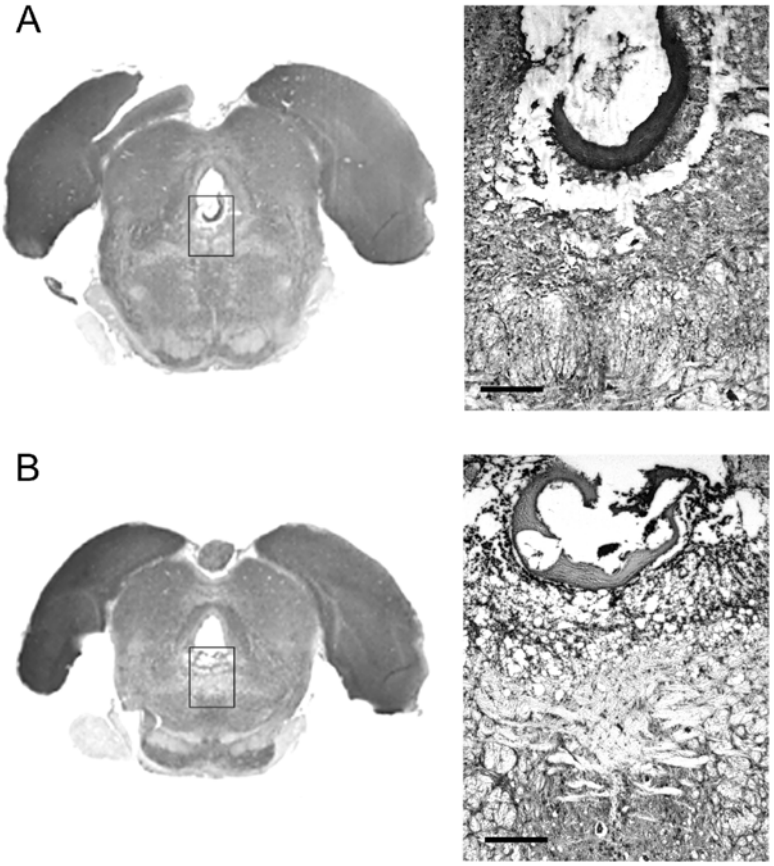

Fig. 4 – Histological sections (10μm thickness), stained by cresyl violet, showing typical lesion of the DRN extending from 7.2 mm (panel A) to 8.3 mm posterior (panel B) to the bregma. Note topographical references around the delimited area. Aq: aqueduct; mlf: medial longitudinal fasciculus; pi: pineal gland; bar calibration, 200μm.

orexigenic response of the brain serotonin-depleted fasted rats. We presume that these conditions the serotonin neurons preserve the ability of decarboxylate the LHTP for synthesis of serotonin. These results possibly mean that serotonergic circuits are recruited during physiological ingestive process for the modulation of appetite intensity. In this context, the participation of the other systems of modulation must not be discarded. In chronic evaluation, the PCPA-treated rats expressed hyperphagic response concomitantly to a gradual increase of gain of body weight. These observations are similar to those reported by Breisch et al. (1976). However, we evidenced a gradual decrease of ingestive levels followed by hypophagy between 25th and 30th day after icv microinjection and posterior normalization. Body weight gain was slowly reverted, achieving normalization from 90th day post-administration of PCPA. Our results suggest that the restoration of ingestive levels and body weight demanded plasticity of the serotonin neurons induced by indoleamine depletion and inhibition of TPO. Possibly, after metabolic clearance of PCPA occurred a resettlement of TPO levels operated by *de novo* synthesis of that enzyme and subsequent production of serotonin. Hypophagy between 25th and 30th day after PCPA microinjection probably reverberate the overexpression of TPO, based on similar conclusions postulated elsewhere (Richard et al. 1990, Lima et al. 2004). Presumably, the normalization of

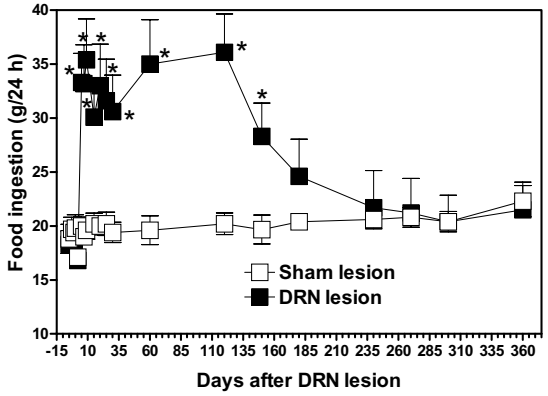

Fig. 5 – Chronic effect of the electrolytic lesion of DRN on the food intake in rats. Data are presented as mean ± standard error. $^{*}P < 0.05$ compared to sham lesion group.

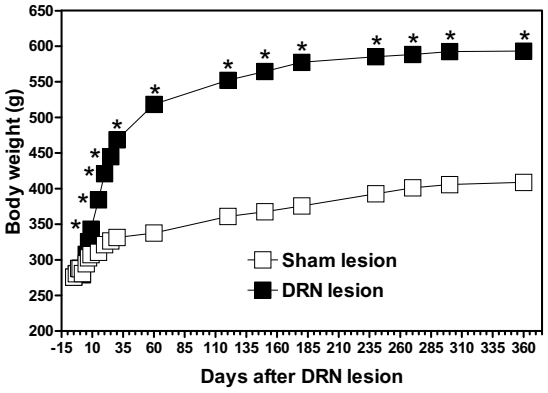

Fig. 6 – Chronic effect of electrolytic lesion of DRN on the body weight in rats. Data are presented as mean ± standard error. $^{*}P < 0.05$ compared to sham lesion group.

the serotonin turnover, and possibly high compensatory expression of other anorexigenic pathways (simultaneously to activation of control systems of the energetic balance), would restore the set point of body weight.

Data concerned to DRN-lesioned rats disclose that suppression of ascending circuits influenced the modulation mechanism of orexigenic activity as well as of energetic balance set point operation and therefore, of body weight adjustment. In this respect our observations parallel with findings regarding lesion of hypothalamic ventromedial nucleus (VMN) (Brobeck et al. 1943, Tepperman et al. 1943, Bray and York 1979, Hallonquist and Brandes 1983, Vilberg and Keesey 1984). These authors referred an intense increase of the food ingestion associated with obesity for long time. Hallonquist and Brandes (1983) showed a gradual decrease of the ingestive levels after 12 weeks post-surgery, however, with preservation of obesity. Data of the current work represent the former study of the feeding behavior in raphe-lesioned rats showing similar feature to those achieved in VMN-lesioned rats. In other papers, the data regarded to hyperphagia *vs* obesity are controversial possibly because the authors employed young rats and, in addition they performed the evaluations for a shorter period. Long time evaluation allows us to evidence either the restoring of food intake levels, 6 months after DRN lesion and the maintenance of high adiposity index. The recovery of the mean values of food ingestion suggests the achievement of a new homeostatic status possibly consequent to orexigenic activity modulatory system plasticity. Future studies shall elucidate which neural circuit arranges that plasticity reaction. Is tempting to hypothesize that negative feedback loop from hypothalamus or originating at peripheral sites would constitute one of the neural substrate disconnected by DRN lesion. This postulation is consonant with recent evidences which orexigenic neurons from lateral hypothalamus project toward DRN where synapse with serotonergic neurons (Janeckova 2001, Rodgers et al. 2002). In this line of reasoning, leptin receptors were identified on DRN serotonergic neurons and, in addition, hypophagic effect of that hormone is partially mediated by serotonergic activity (Collin et al. 2000, Finn et al. 2001, Telles et al. 2003, Yamada et al. 2003).

Our data are similar to those reached with VMN lesion in original studies of Brobeck et al. (1943) and Tepperman et al. (1943) and, reproduced later by Bray and York (1979), Hallonquist and Brandes (1983) and Vilberg and Keesey (1984). It is known at moment that among multiple disturbances caused by VMN lesion one of them is the disconnection between peripheral signal of leptin and mRNA ex-

pression of neuropeptide Y (NPY). NPY represents the main convergence pathway of the orexigenic behavior (Hillebrand et al. 2003, Kalra et al. 2003, Kalra and Kalra 2003). Briefly, daily rhythm of mRNA NPY demands the integrity of VMN (Dube et al. 1999). The VMN coordinately with hypotalamic arcuate (AN) and dorsomedial (DMN) nuclei constitutes a circuitry responsive to leptin feedback which regulates the caloric ingestion and adjusts the energetic content of the adipose tissue (Bernardis and Berllinger 1998). Hassanain and Levin (2002) demonstrated in this context that fasted diet-induced obese (DIO) rats showed a 53% greater reduction in the ventromedial nucleus turnover than fasted diet-resistant rats. Thus, DIO-prone rats show abnormalities in brain serotonin turnover which may predispose them to become obese when dietary fat and caloric density are increased. These observations strengthen the findings of De Fanti et al. (2000) that showed a low capacity of serotonergic transmission from DRN toward VMN in Zucker rats, genetically obese. In this line, results of current study are evidences that serotonergic pathways are implicated with the acute control of food ingestion and chronically involved with the mechanisms of energetic balance set point adjustment and, therefore, with body weight regulation. These conclusions are based on (i) in the acute restoring (partially at least) of the appetite modulation in serotonin-depleted and –fasted rats induced by LHTP and, (ii) in the maintenance of obesity for long time in DRN-lesioned rats despite of the normalization of food ingestion.

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RESUMO

O papel do sistema serotonérgico no comportamento alimentar foi avaliado através de lesões eletrolíticas do núcleo dorsal da rafe (L-NDR) e da administração de para-clorofenilalanina (PCPA, 3 mg/5 μl, icv). Avaliações crônicas foram realizadas durante 120 e 360 dias em ratos injetados com PCPA e L-NDR, respectivamente. Avaliações agudas foram realizadas em ratos em jejum e injetados com PCPA e l-triptofano (LHTP, 30 mg/kg, ip). Ratos lesionados apresentaram um aumento de 22-80% na ingestão de alimento até o sexto mês enquanto a obesidade foi evidenciada e mantida por todo o período. Ratos injetados com PCPA apresentaram um aumento da ingestão alimentar seguido de uma hipofagia do 25° ao 30° dia e um aumento transitório do peso corporal do 5° ao 60°. Agudamente, o LHTP reverteu parcialmente o aumento da ingestão de alimento em ratos tratados com PCPA e jejuados, sugerindo a preservação da capacidade de descarboxilação do precursor pelos neurônios serotonérgicos. A lenta recuperação dos níveis de ingestão alimentar em ratos lesionados revela um mecanismo de neuroplasticidade dos sistemas de regulação do comportamento alimentar. Estabelecimento de platô na curva de peso corporal dos ratos lesionados representaria o estabelecimento de um novo e mais elevado ponto de calibração do balanço energético.

Palavras-chave: ingestão de alimento, sistema serotonérgico, núcleo dorsal da rafe, lesão eletrolítica, para-clorofenilalanina, obesidade.

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