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## Galanin in the PVN increases nutrient intake and changes peripheral hormone levels in the rat

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### Abstract

In self-selection feeding paradigms, rats display differential patterns of nutrient (protein, carbohydrate or fat) intake. Factors known to influence this selection include brain peptides as well as circadian parameters. In this series of experiments we investigated the role of PVN galanin in nutrient intake during the early and late dark periods in the rat. Rats were allowed to select between three isocaloric diets enriched in protein, carbohydrate or fat. Following a 2-week adaptation period, the animals' 24-h intake was monitored for 4 weeks. Galanin was injected into the PVN and food intake was measured 1, 2 and 24 h post-injection. Galanin significantly increased the 1 h total food intake but it failed to increase the intake of any particular nutrient. Galanin had no effect 2 or 24 h post-injection. Analysis of the data grouped by preference based on the rats 24 h baseline selection patterns over the 4-week period revealed that galanin seem to increase the preferred nutrient. That is, galanin preferentially increased the intake of the carbohydrate- or fat-rich diet in animals with high (over 40% of the total food intake) 24-h baselines in this particular nutrient. Finally, analysis of the plasma hormone levels after paraventricular galanin administration revealed a significant increase in noradrenaline levels, a small reduction in plasma insulin with no effects on adrenaline, glucose or corticosterone. The data revealed that galanin in the PVN influences both food intake and metabolic functioning. PVN galanin significantly increases sympathetic outflow and seems to stimulate the intake of the individual rat's preferred macronutrient.

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**Keywords:** Galanin; Food intake; Nutrient preference; Fat; Carbohydrate; Paraventricular; Hypothalamus; Sympathetic nervous system; Noradrenaline; Insulin; Norepinephrine

### 1. Introduction

Galanin is an orexigenic peptide known to stimulate food intake in satiated rats after paraventricular hypothalamic administration [26]. Studies using the macronutrient self-selection paradigm in rats have shown this peptide to be particularly effective in stimulating the consumption of fat, especially at the end of the nocturnal (active) cycle [18,28]. However, this notion of galanin as a fat stimulatory agent was challenged by studies which failed to demonstrate a specific increase in fat following PVN galanin administration [3,7,21,22]. According to Crawley

[4], galanin increases ingestion relatively independently of macronutrient content. Taken together the available data in literature suggest that there might be a specific effect of galanin on nutrient preference, but this effect might be strongly dependent on the type of self-selection paradigm, the animals' individual preference and the time of the day. In our lab, we use a self-selection paradigm with isocaloric enriched diets that contain only small differences in nutrient content, allowing to dissociate nutrient preference from energy content. The major aim of this study was therefore to determine whether galanin has a role in the intake of fat or any other particular nutrient in this particular experimental set-up.

Most hypothalamic neuropeptides that are known to influence food intake and/or macronutrient choice are also involved in the regulation of peripheral energy metabolism [1]. An example is neuropeptide Y (NPY), a neuropeptide that not only selectively stimulates carbohydrate intake, but also decreases sympathetic

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\* This article is based on studies, performed by Dr Kyrkouli during her sabbatical in Groningen. Vivi Kyrkouli passed away on May 18th, 2006. She was a great friend and colleague and will be greatly missed.

activity, stimulates insulin and corticosterone release and increases parasympathetic activity in resting conditions and during feeding [29]. The effects of hypothalamic galanin administration on peripheral energy metabolism are relatively unknown. The only data available suggest that central galanin administration may increase plasma corticosterone levels [26] and reduce sympathetic activity [11]. Therefore, the second aim of this study was to investigate whether hypothalamic galanin administration may influence the hormonal and metabolic factors that are known to be involved in the regulation of peripheral energy metabolism.

## 2. Methods

### 2.1. Subjects and surgery (1)

Thirteen adult, male Wistar rats, weighing 275–300 g at the time of surgery, were housed individually in plexiglass cages (25 × 25 × 30 cm), in a temperature-controlled (22 °C) room under a 12:12 h light–dark cycle. Subjects were stereotaxically implanted with bilateral 30-gauge stainless-steel guide cannulas, 0.5 mm apart, targeted on the dorsal surface of the PVN. With the incisor bar 3.3 mm below the interaural line, the stereotaxic coordinates used were: 7.0 mm anterior to the interaural line and 7.5 mm ventral to the dura. The rats were allowed 2 weeks to recover from surgery before the experiments started.

### 2.2. Diets

Animals were maintained on a freely feeding self-selection paradigm with three enriched diets, protein, carbohydrate and fat. The diets consisted of 75% rat chow (RMH-B meel, Hope Farms, The Netherlands) and 25% protein (casein, Merck), carbohydrate (glucose, Merck) or fat (lard) (% by energy content). The diets were offered simultaneously in three dishes. Animals were provided with fresh food daily at CT 11. Total 24 h intake of each diet was measured five times/week for 10 weeks. The position of the dishes in the cage changed everyday to prevent the formation of position preferences. Subjects were maintained ad lib on these diets for at least 10 days prior to cannula implantation to stabilize their baseline feeding patterns.

### 2.3. Galanin treatment and nutrient intake

Two weeks after surgery drug tests were initiated. Tests were initiated at circadian time 12 (CT 12), immediately before lights off for the early dark condition, and at CT 22, 2 h before lights on for the late dark condition. The food cups were removed and weighed and the animals were injected in the PVN with galanin (Peninsula Laboratories, 1 µg/side) or vehicle. The vehicle was sterile artificial cerebrospinal fluid (aCSF) containing NaCl 122 mM, KCl 3.1 mM, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.3 mM, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 0.4 mM and MgCl<sub>2</sub>·6H<sub>2</sub>O 1.2 mM. The compounds were infused in a volume of 1 µl/side during a 5-min period through a pump. Every rat was tested twice, once for galanin and once for aCSF (in random order with at least 1 week between consecutive tests). Food cups with fresh food were immediately returned and food intake was measured 1, 2 and 24 h post-injection.

### 2.4. Subjects and surgery (2)

A second experiment was performed in eight rats. After the termination of the feeding studies, these rats underwent a second surgery in which they were provided with a silicon catheter in the jugular vein. Catheter placement was performed according to Steffens [24], with the tip of the catheter positioned at the entrance of the right atrium. The other end was externalized on top of the skull, where they were fixed with acrylic cement. The jugular vein catheter allows frequent sampling of well-mixed central venous blood in unanesthetized, undisturbed, freely moving rats. The rats were allowed 2 weeks to recover before the experiments started.

### 2.5. Galanin treatment and neurohormonal responses

In the second experiment, blood samples were taken before, during and after introhypothalamic galanin infusion to obtain information on the hormonal and metabolic factors that control peripheral energy balance. The experiments were performed in the light period between CT 2 and CT 6. On the experimental day, food was removed after lights went on. At CT 2, the venous catheter and brain cannulas were connected to polyethylene tubings to enable remote venous access and brain infusion. After a resting period of at least 1 h, two baseline blood samples were taken, with a 10-min interval. Then, a bilateral infusion into each PVN was started, which ran at a rate of 1 µl/min over a period of 2 min. Two infusions were performed, in random order. Galanin was infused at a rate of 0.5 µg/min. Control treatment consisted of an infusion of a similar amount of sterile artificial CSF. Blood samples of 0.45 ml were taken throughout and after the infusion period to measure the changes in blood glucose and plasma insulin, noradrenaline, adrenaline and corticosterone at the sampling time points  $t = -11, -1, 5, 10, 15, 23, 30, 45$  and 60 min. After each blood sample the same amount of donor blood was given to avoid changes in hemodynamics.

The blood samples were collected in tubes containing EDTA and kept chilled during the experiment. Afterwards, 50 µl blood was removed to determine blood glucose levels, while the remaining blood was centrifuged for 15 min at 2600×g. Plasma aliquots were frozen until determination of levels of insulin (insulin RIA Kit, Linco Research, St. Charles, MO, USA), adrenaline and noradrenaline (HPLC with electrochemical detection), and corticosterone (HPLC with UV detection). Glucose samples were analyzed by the ferricyanide method of Hoffman in a customized Skalar Auto analyzer (Skalar, Breda, The Netherlands). Blood glucose levels were measured at all time points, plasma insulin at  $t = -1, 5, 10, 15, 30$  and 60 min, catecholamines at  $t = -1, 5, 10, 23$  and 45 min and corticosterone levels at  $-11, 15$  and 45 min.

### 2.6. Histology

At the end of the experiments, the rats were deeply anaesthetized with sodium pentobarbital (90 mg/kg i.p.) and perfused intracardially with saline followed by 4% paraformaldehyde solution. Brains were cut in 40 µm coronal sections, stained

with cresyl violet, and histologically analyzed for placement of the cannula. In two cases, the cannulas were placed at the posterior end of the PVN and these animals were excluded from the final analysis.

## 2.7. Statistics

The data were evaluated using a multiple analysis of variance with repeated measures (MANOVA) followed by Duncan's multiple range test. Additional direct comparisons were made using Student's *t*-test. Relationships between 24 h food intake and responses to GAL injection were examined using Pearson's correlation analysis.

## 3. Results

### 3.1. 1 h diet intake

As depicted in Fig. 1 (top level), during the early dark period, 1 h following aCSF infusion, rats consumed a total of 6.6 kcal, from which 2.8 kcal (42%) in carbohydrate-enriched form, 1.9 kcal (29%) in protein-enriched form and 1.9 kcal (29%) in fat-enriched form. During the late dark period, the total calories consumed were 4.4 kcal, significantly less than those in the early dark [ $F(1,40)=5.34, p<0.03$ ]. In the late dark, rats consumed 1.8 kcal (40%) of protein-enriched diet, 1.35 kcal (30%) of carbohydrate-enriched diet and 1.35 kcal (30%) of fat-enriched diet.

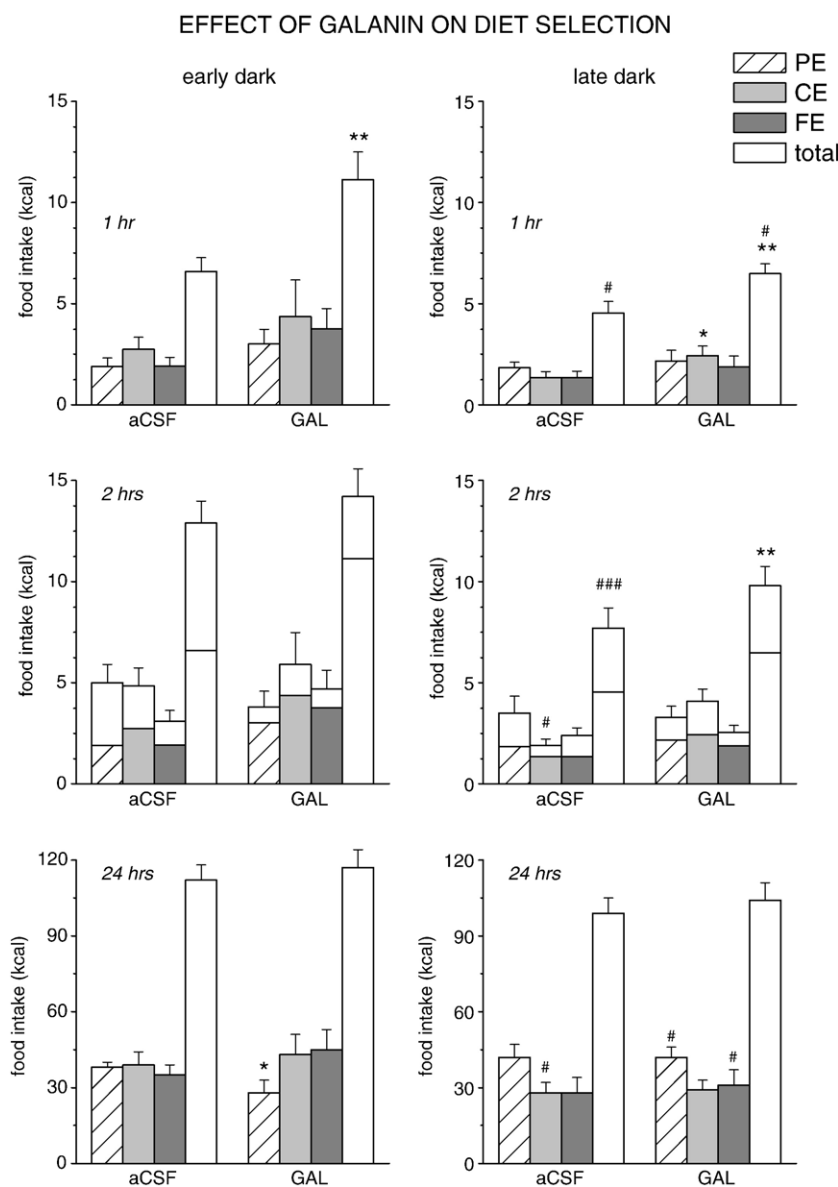


Fig. 1. Effect of infusion of aCSF or galanin into the paraventricular hypothalamus on diet selection and total food intake. PE = protein enriched, CE = carbohydrate enriched, FE = fat enriched. Left panels: infusion at CT 12, right panels: infusion at CT 22. Upper panels: food intake in the 1st hr. Middle panels: food intake during the 2nd hr (stained bars) and total 2 hr intake (complete bars). Lower panels: total food intake in the 24 hrs after injection. N=11, \* indicates a significant difference between galanin and aCSF administration, # indicates a significant difference between late dark and early dark.

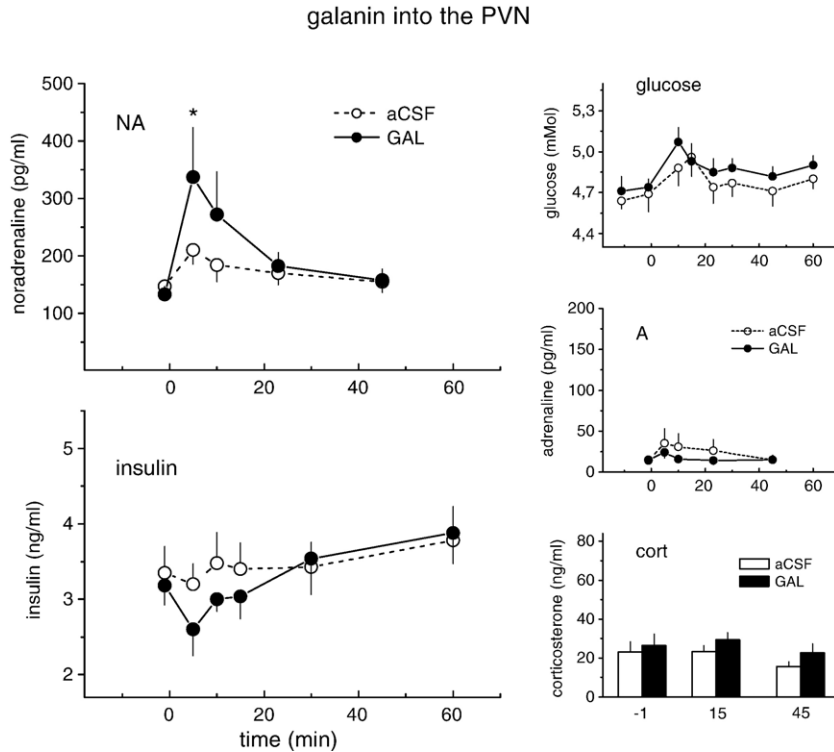


Fig. 2. Blood glucose and plasma levels of noradrenaline (NA), adrenaline (A), insulin and corticosterone after infusion of 1µg galanin (bilaterally) or aCSF into the PVN. N=8 rats. Filled symbols (●) = Galanin. Open symbols (○) = aCSF.

Overall analysis of the effect of galanin infusion into the PVN (GAL) on nutrient-enriched diets by GLM 3-way ANOVA (time versus treatment versus diet) revealed a significant effect of time [ $F(1,120)=6.83$ ,  $p<0.01$ ] and of treatment [ $F(1,120)=6.39$ ,  $p<0.01$ ]. During the early dark period, PVN GAL infusion caused an approximately 70% increase in total food intake [+4.5 kcal,  $F(1,40)=8.66$ ,  $p<0.01$ ]. There was no significant increase in the intake of any particular nutrient-enriched diet. In the late dark period, the effect of GAL on nutrient enriched diets was significantly smaller as compared to the effect of this peptide in the 1st hour of the dark [45% increase,  $F(1,40)=6.74$ ,  $p<0.02$ ]. During that period, PVN GAL infusion resulted in a significant increase of total food intake [+2.0 kcal,  $p<0.01$ ]. A two-tailed *t*-test showed a significant increase in carbohydrate-enriched diet intake ( $t=2.44$ ,  $p<0.03$ ).

### 3.2. 2 h diet intake

The second-hour food intake as well as the cumulative 2-hour food intake can be seen in Fig. 1 (middle level). During the early dark period, the 2nd hour following aCSF infusion, rats consumed a total of 6.2 kcal, of which 3.1 kcal (50%) in protein-enriched form, 2.1 kcal (34%) in carbohydrate-enriched form and 1.0 kcal (16%) in fat-enriched form. During the late dark period, the total calories consumed were 3.4 kcal, significantly less than those in the early dark [ $F(1,40)=21.74$ ,  $p<0.001$ ]. In the 2nd hour of the late dark, rats consumed 1.7 kcal (50%) of protein-enriched diet, 1.1 kcal (32%) of fat-enriched diet and 0.6 kcal (18%) of carbohydrate-enriched diet.

### 3.3. 24 h diet intake

As can be seen in Fig. 1, when animals were provided with fresh food in the early dark, 24 h following an aCSF infusion, rats ( $n=11$ ) consumed a total of 114 kcal of which 38 kcal (33%) in protein-enriched form, 41 kcal (36%) in carbohydrate-enriched form and 35 kcal (31%) in fat-enriched form. When rats were provided with fresh food in the late dark, the total calories consumed were 99 kcal, slightly less than those in the early dark. Specifically, rats ingested 42 kcal (42%) in protein-enriched form,

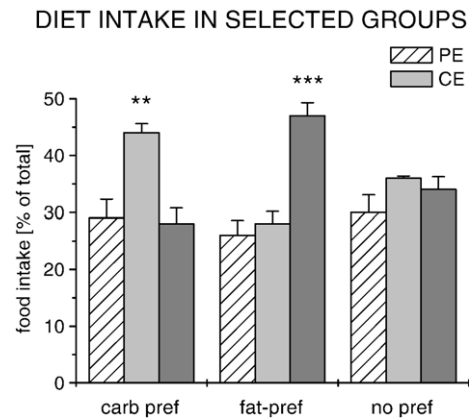


Fig. 3. Diet intake in rats ( $n=11$ ) grouped on their preference based on the 24 hrs baseline selection patterns over a 4-week period. PE = protein enriched, CE = carbohydrate enriched. Dark solid bar represents intake of the fat enriched diet. N=11, \* indicates a significant difference between the preferred and non-preferred diet.

28 kcal (29%) in carbohydrate-enriched form and 29 kcal (29%) in fat-enriched form. The carbohydrate-enriched diet consumed during the late dark was significantly less as compared to that ingested during the early dark ( $t=2.22, p<0.05$ ). During neither time period, PVN GAL infusion altered the total amount of food ingested. In the early dark, however, GAL significantly reduced protein-enriched diet intake ( $t=2.51, p<0.03$ ).

3.4. Effects on hormone levels

The effect of PVN GAL infusion ( $n=8$ ) on plasma levels of glucose, catecholamines, insulin and corticosterone are depicted in Fig. 2. Galanin significantly enhanced plasma noradrenaline

levels above control levels [ $F(4,33)=2.73, p<0.05$ ], 5 min following the infusion ( $p<0.05$  by Duncan’s multiple range test). At the same time point, plasma insulin levels were somewhat but not significantly reduced in the galanin treated animals.

3.5. Analysis of the diet intake data grouped by preference

Over the course of the first study it became apparent that there were individual differences in nutrient-enriched diet preference among animals. To study whether these individual preferences had an effect on GAL-induced food intake we separated the rats in three groups based on their baseline preference for specific diets. Diet preference was defined as more than 40% intake of a specific

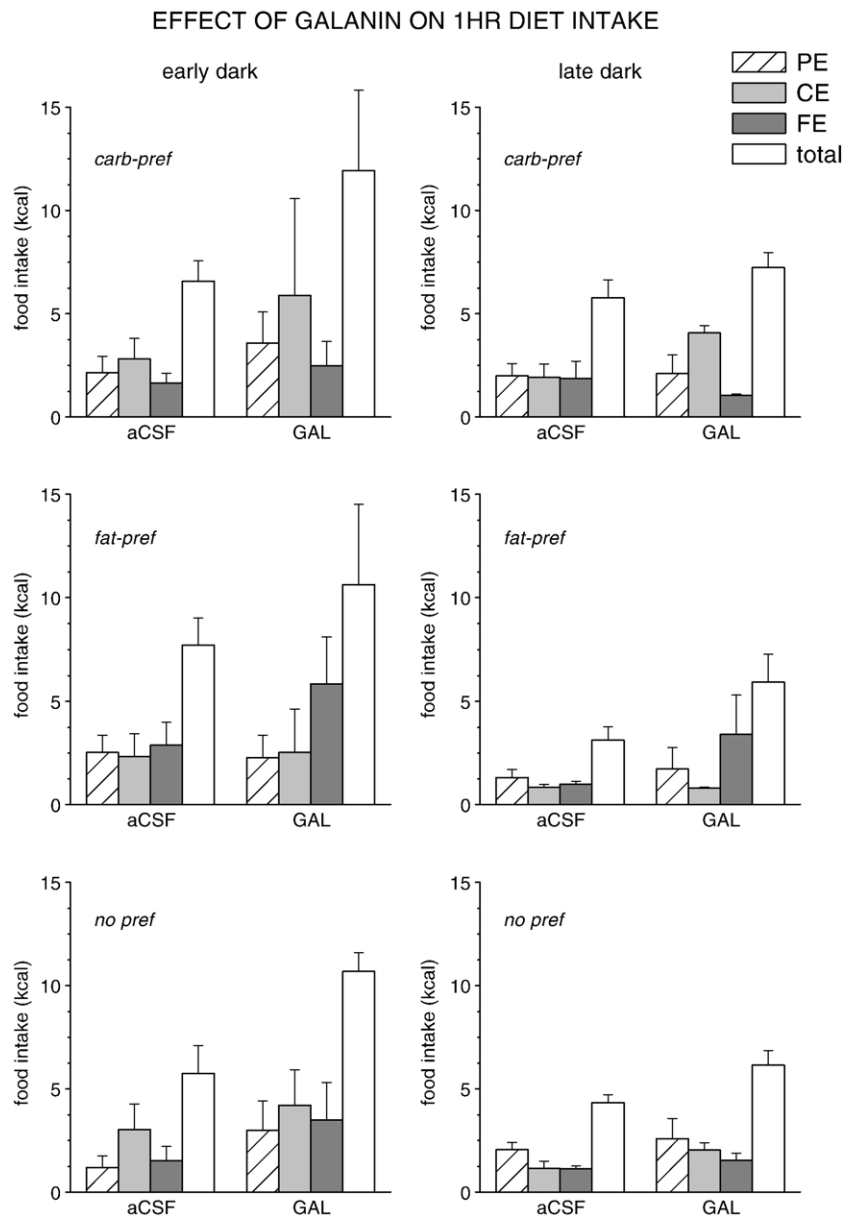


Fig. 4. Effect of infusion of aCSF or galanin into the paraventricular hypothalamus on diet selection and total food intake. PE = protein enriched, CE = carbohydrate enriched, FE = fat enriched. Left panels: infusion at CT 12, right panels: infusion at CT 22. Upper panels: data from carbohydrate preferring rats. Middle panels: data from fat preferring rats. Lower panels: data from rats without a specific preference. N=11.

enriched diet over a period of 4 weeks. Fig. 3 shows the percentage of each diet consumed in 24 h, over a 4-week period, of rats with a strong preference for either carbohydrate ( $n=4$ ) or fat ( $n=3$ ) and rats with no preference for a specific nutrient-enriched diet ( $n=4$ ). Analysis with repeated measures ANOVA revealed a significant difference between the three groups in diets ( $F(2,16)=6.37, p<0.01$ ) and an interaction between groups and diets [ $F(4,16)=7.82, p<0.002$ ]. One way ANOVA showed a significant difference between the three diets for the group with a strong preference for carbohydrates [ $F(2,9)=10.78, p<0.01$ ] and the group with a strong preference for fat [ $F(2,6)=12.24, p<0.002$ ].

The effects of PVN GAL on 1 h diet intake in the three groups of rats during the early and late dark periods are shown in Fig. 4. Analysis by GLM 4-way ANOVA (treatment versus time versus preference versus diet) revealed a significant difference in treatment [ $F(1,96)=9.08, p<0.003$ ] and time [ $F(1,96)=7.65, p<0.007$ ]. There was also a two-way interaction between preference and diet [ $F(4,96)=4.07, p<0.004$ ] as well as a three-way interaction between treatment, preference and diet [ $F(4,96)=2.70, p<0.03$ ]. Due to the small number of rats per group, however, one-way ANOVA analyses did not quite reach statistical significance.

#### 4. Discussion

These results demonstrate that in rats fed a nutritionally complete diet, PVN GAL does not stimulate the intake of any particular nutrient neither in the early nor in the late dark. Rather, GAL seems to stimulate the intake of the preferred nutrient-enriched diet in rats with baseline preferences for carbohydrate or fat. This effect is more pronounced in the late dark.

Galanin increased 1-h total food intake, both in the early and the late dark periods. This is consistent with previous studies on PVN GAL administration [21]. It is generally accepted that food intake in the early dark has a function to restore reduced energy stores [2,9], while food intake in the late dark is more anticipatory in nature [23,25]. In this study, the significant increase in food intake produced by GAL during the first hour of the early dark was compensated for by a lack of further increase in eating during the second hour. In the late dark, however, the rats continued to eat during the second hour, thus, significantly enhancing their 2-h total food intake.

Galanin's failure to stimulate fat intake is consistent with several studies on GAL and diet selection [7,14,21]. Using a fat-chow choice paradigm, Corwin et al. [3], found no significant increase in fat intake following PVN GAL administration (early dark, vegetable shortening, no baselines). In a two-choice composite diet as well as a three choice macronutrient diet, Smith et al. [22] reported that the feeding stimulation produced by GAL was not diet dependent. In a protocol with an anti-galanin antisense oligonucleotide, a similar lack of effect of GAL on fat intake was reported [6].

These findings are in contrast to earlier studies on the effect of GAL on macronutrient selection [9,18,26,28]. According to these studies, GAL preferentially enhances fat intake, especially during the late dark period. The reason for this discrepancy is not clear. It

does not seem to be due to the use of lard versus vegetable shortening as has been previously suggested [22] because, in this study, we also used lard but did not see the increase in fat intake. Similarly, it cannot be due to the kind of diet used (macronutrients versus a nutritionally complete diet) because Smith et al. [21] also used macronutrients but failed to see an increase in fat intake following PVN GAL administration in tests that were, however, conducted in the middle of the light period.

A more likely explanation could involve the rats' natural dietary preferences. Studies have demonstrated that rats exhibit considerable individual differences in their choice of macronutrients, especially carbohydrate and fat [19,20]. This variability in dietary choice has been documented so far in Sprague–Dawley rats fed a pure macronutrient diet. As stated previously in Methods, during data analysis, it became apparent that many animals showed a particular preference for a specific nutrient-enriched diet. This way we could dissociate three groups of rats based on their individual preference for a specific nutrient over a 24-h period: carbohydrate preferrers, fat preferrers and animals that ate equal amounts of each diet. This was particularly interesting considering the observation that all animals were consistent in their preference over a 4-week period. After selecting the groups, the data revealed that galanin infusion selectively stimulated carbohydrate intake in carbohydrate-preferring rats and fat intake in fat-preferring rats 1 h post-injection. Although these differences did not quite reach statistical significance in the early dark period due to a small number of animals per group, these data are highly suggestive for an action of paraventricular galanin to enhance the intake of the preferred nutrient. It might be hypothesized that hypothalamic galanin release may reflect the rewarding properties of food, which is supported by the observations by Rada et al. that hypothalamic galanin initiates feeding (and ethanol intake), at least in part, by activating the mesolimbic dopaminergic system [12,13].

Galanin administration into the PVN caused an increase of plasma NA with no change in A reflecting a selective activation of the neuronal branch of the sympathetic nervous system [16]. This increased sympathetic activity is accompanied with a small (but not significant) reduction in plasma insulin levels. This effect, increased sympathetic outflow and reduced insulin release, is remarkably similar to the effect of galanin in the periphery where it is co-released together with NA by the sympathetic nerve terminals, especially those that innervate the  $\beta$ -cells of the endocrine pancreas [5,15]. At the pancreatic  $\beta$ -cell, galanin selectively inhibits insulin release without an effect on cardiovascular functioning [10]. It is tempting to speculate that galanergic pathways in the brain may be involved in the selective activation of galanin-containing nerves at the level of the pancreas. The data from the present study are in contrast with an earlier study from Nagase et al. [11] who showed that third ventricular administration of galanin reduced sympathetic activity to interscapular brown adipose tissue, consistent with the hypothesis that food intake and sympathetic nervous system activity have a reciprocal relationship. The reason for this difference remains to be investigated.

Plasma corticosterone levels remained unchanged after galanin administration which means that a reduction in HPA-axis activity, as seen by the group of Leibowitz [27], did not occur. Instead, previous data from our lab suggest that the hypothalamic

regulation of HPA-axis activity is mainly mediated by noradrenergic and serotonergic neurons acting on  $\alpha_2$ -adrenoceptors and 5-HT<sub>1a</sub>-receptors in the PVN [8,17].

In conclusion, the data of the present study reveal that galanin in the PVN influences both food intake and metabolic functioning. PVN galanin significantly increases sympathetic outflow and seems to stimulate the intake of the individual rat's preferred macronutrient.

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