Chapter 6

Differential effects of rAAV-mediated NPY overexpression in the PVN and LH on energy balance

Birgitte Tiesjema, Susanne E. la Fleur, Mieneke C.M. Luijendijk, Andries Kalsbeek, Roger A.H. Adan

DIFFERENTIAL EFFECTS OF RAAV-MEDIATED NPY OVEREXPRESSION IN THE PVN AND LH ON ENERGY BALANCE

ABSTRACT

The PVN and LH/PFA are in varying degrees involved in the acute, hyperphagic effects of NPY. Nevertheless, implication in energy expenditure and long-term effects of NPY are less understood. In order to further clarify the role of the PVN and the LH/PFA in NPY-induced obesity, we injected AAV-NPY in the PVN or LH/PFA of adult rats. Animals were then followed for 50 days.

Although injections in both areas resulted in obesity, caused by increased food intake and decreased energy expenditure, clear differences were observed in the manner via which obesity develops. Whereas food intake and body weight gain of PVN-NPY rats was only temporarily increased, LH-NPY rats remained hyperphagic for the entire 50 days. Additionally, AAV-NPY injections in the PVN only increase meal frequency, while LH/PFA injections alter both frequency and size. Moreover, in LH-NPY rats, but not PVN-NPY rats, circadian rhythmicity with regard to food intake and body temperature was lost. These data suggest that the NPY system differentially regulates energy intake and energy expenditure in the PVN and LH/PFA, which together adjust energy balance.

INTRODUCTION

Neuropeptide Y (NPY) is widely expressed in the brain and is involved in several aspects of the regulation of energy homeostasis. Besides having a strong, positive effect on food intake, NPY is also able to reduce metabolic rate and thermogenesis (193;206;208;220;335). Chronic intracerebroventricular (ICV) infusion of NPY results in obesity, characterized by hyperphagia, increased lipogenesis in liver and adipose tissue and elevated plasma concentrations of leptin, insulin and corticosterone (121;201;203;212;214).

NPY neurons involved in energy balance are mainly found in the arcuate nucleus (Arc), and project to a variety of areas, including the paraventricular nucleus of the hypothalamus (PVN) and the lateral (LH) / perifornical area (PFA). Also the dorsomedial nucleus of the hypothalamus (DMH) and medial preoptic area receive NPY-ergic terminals from the Arc (56;177;178). Most studies on the local role of NPY have focused on the PVN, due to the high amount of NPY-ergic terminals that are present in that nucleus (178). Nevertheless, the strongest feeding response is observed after injection of NPY into another area, i.e. the PFA.

Although these nuclei are in varying degrees involved in the acute hyperphagic effects of NPY, implication in energy expenditure and long-term effects of NPY are less understood. The acute effects of NPY in the PVN on thermogenesis are contradictory; both increases and decreases in body temperature were reported (204;205;223). In addition, the thermogenic response to a single injection in the LH fluctuates with the dose of NPY administered, with hyperthermia observed after low doses, and hypothermia after higher doses (204;205).

Considering the diversity in effects of NPY infusion into the PVN and LH/PFA on energy expenditure, chronic elevated NPY in the PVN or LH probably also differentially regulate other parameters related to obesity.

So far, the involvement of different brain regions in the effects of NPY on energy balance was investigated using single (PVN and LH) or repeated injections (PVN) of NPY locally in the brain. In order to understand which brain regions are involved in induction of obesity by increased NPY signaling, it is essential to continuously increase NPY locally in the brain, which is not feasible using infusions of ligands. We have used viral mediated overexpression of NPY to further unravel the specific role of the PVN and LH (including the PFA) in NPY-induced obesity. Recently, it was shown that when NPY is overexpressed with this technique at the adult stage in the whole hypothalamus of mice, obesity is induced (253). In the present study, the long-term effects of NPY overexpression in either the PVN or LH were compared with regard to feeding behavior (including meal patterns), body temperature and locomotor activity. In addition, the site-specific effects of increased NPY signaling on endocrine parameters and body composition were compared.

MATERIAL AND METHODS

Animals

Male Wistar rats weighing 220-250 g were purchased from Charles River (Crl-Wu, Germany). They were individually housed in filtertop cages with *ad libitum* access to food and water. Animals were kept in a temperature and humidity controlled room (21±2 °C) under a 12h/12h light/dark cycle (lights on at 0600 h). All experimental procedures were approved by the Committee for Animal Experimentation of the University of Utrecht, Utrecht, The Netherlands.

Surgical procedures

Rats were anesthetized with 0.1 ml/100 g hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.05 ml/100 g ip dormicum (Hoffman-LaRoche, Mijdrecht, The Netherlands). Transmitters (TA10TA-F40 Data Science International, St Paul, Minnesota, USA) were placed in the abdominal cavity. Rats were left to recover for three weeks.

Seven days after baseline recordings, rats were anesthetized again as described above. Using a stereotax, rAAV-NSE-NPY (n=12, LH-NPY) or rAAV-NSE-empty (used as control) (n=7, LH-contr) was injected bilaterally into the LH (coordinates AP: -3.0 mm from bregma, ML: ±1.6 mm from bregma, and DV: -8.6 mm below the skull). In a second experiment, rats were injected bilaterally into the PVN (coordinates AP: -1.8 mm from bregma, ML: ±0.3 mm from bregma, and DV: -8.1 mm below the skull, rAAV-NSE-NPY n=16 (PVN-NPY), rAAV-NSE-empty n=12 (PVN-contr)). rAAV-NPY and rAAV-empty were a kind gift of M.J. During (New York). Production of rAAV-NPY has been described

previously (339;340). 1 µl of virus (1×10^8 genomic copies) was injected per site over five minutes, after which the needle was kept in place for ten minutes before removal. After each surgery, rats received an injection with 5 mg/kg carprofen (Vericore Ltd, Dundee, United Kingdom).

Data analysis

One week before, until fifty days after viral injections, body weight, food intake, body temperature and locomotor activity were recorded.

Body weight gain and food intake were measured daily at 11.00h. Body temperature and locomotor activity were automatically recorded via the transmitters that send digitized data via radio frequency signals to a nearby receiver. The data were recorded every ten minutes using DSI software (DSI, St Paul, MN). On day 21 and 48 meal patterns were recorded. Food hoppers were weighed automatically and data were send to a computer every 12 seconds for 24 hours. A meal was defined as an episode of food intake with a minimal consumption of 0.5 g chow, and an inter-meal interval of 5 minutes.

Collection of blood and tissues

At day 50 after injection of the virus, rats were decapitated in the morning, trunk blood was collected in heparinized tubes after adding 83 µmol EDTA and 1 mg aprotinin, and immediately placed on ice. Plasma samples were stored at -20 °C until further analysis.

Brains were immediately removed after decapitation, quickly frozen in cold isopentane (-35 °C) and stored at -80 °C. Retroperitoneal, epididymal, mesenteric and subcutaneous white adipose tissue (WAT) was isolated and weighed. Pituitaries, adrenals and the thymus were also isolated and weighed.

Quantitative in situ hybridization

16 µm coronal sections were used for raISH. 33P-labeled antisense RNA probes were made for AgRP, NPY, POMC, MCH and prepro-orexin. raISH procedure has previously been described (345). Expression of AgRP, NPY and POMC in the arcuate nucleus and expression of MCH and prepro-orexin in the LH was quantified using Image J software (National Institutes of Health, Bethesda, Maryland, USA). Expression of NPY was also measured in the PVN and LH to confirm viral-mediated overexpression.

Plasma analysis

Plasma leptin, insulin and corticosterone were analyzed in duplicate using radioimmunoassay kits, (Linco Research, St Charles Missouri, USA for leptin and insulin, ICN Biochemicals, Costa Mesa, California, USA for corticosterone). Plasma adrenocorticotropic hormone (ACTH) was measured in duplicate using a specific rabbit

antiserum directed to the midportion of ACTH, which was kindly provided by Dr G.B. Makara (Budapest, Hungary). Synthetic human ACTH$_{(1-39)}$ (Peninsula Laboratories, Belmont, California, USA) was labeled with ^{125}I and used as tracer (342). Plasma glucose was measured in triplicate using a Glucose/GOD-Perid method (Boehringer Mannheim, Germany).

Statistical analysis

Data are presented as group means ± SEM. Differences in body weight and food intake were assessed using repeated measure analysis. When significant overall interactions were found, post hoc analyses were performed with T-tests. Further statistic analysis was performed with T-tests. Differences were being considered significant at p<0.05.

RESULTS

Verification of AAV injections

Correct injection of AAV-NPY was analyzed by radioactive NPY in situ hybridization. Rats were only included in the study when NPY expression was observed bilaterally in the PVN or LH (Fig. 1), animals with incorrect injections were excluded from analysis (in total, two rats from PVN-contr, three rats from LH-NPY, one rat from LH-contr and seven rats from LH-NPY were excluded, due to unilateral WPRE expression or expression not restricted to the LH).
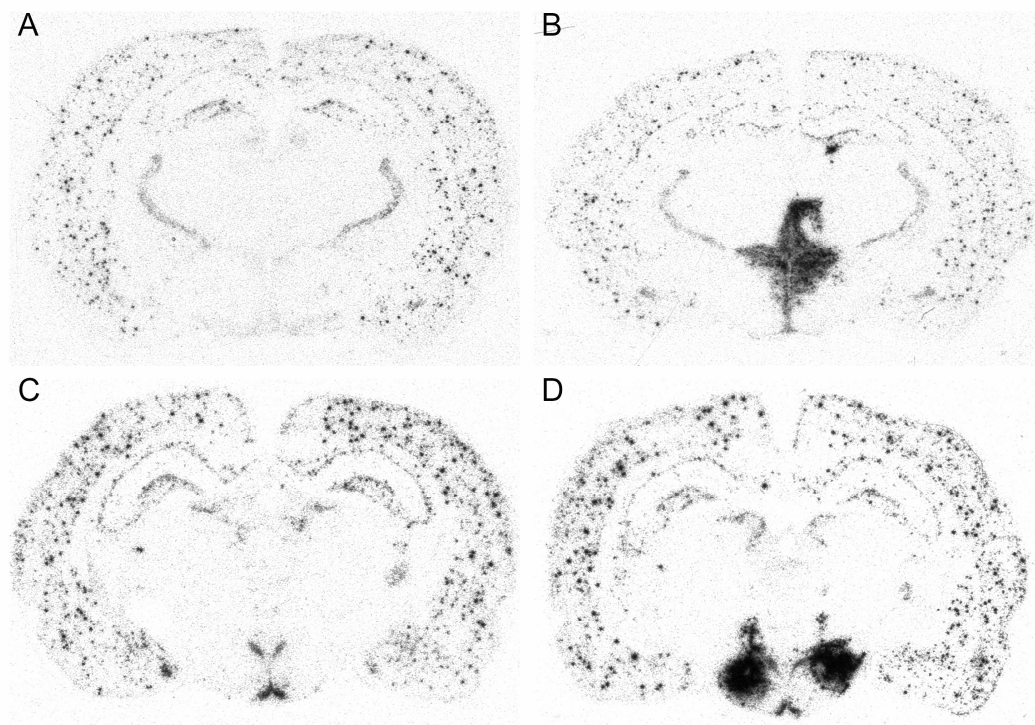
Figure 1: NPY mRNA expression in rats injected with rAAV-empty in the PVN (A) or LH (B), or with rAAV-NPY in the PVN (C) or LH (D).

### AAV-NPY induced effects on body weight and food intake

The first days following injection of the viral particles, all groups of rats showed a similar decrease in body weight gain and intake of food and water. All animals recovered within one week after injection. Both PVN-NPY rats and LH-NPY rats showed an increase in body weight gain from day 9 post injection when compared to controls (Figs 2A and B). In the last ten days of the study body weight gain of PVN-NPY rats was not significantly different from PVN-controls, however, body weight gain of LH-NPY rats remained increased until the end of the study. At day 50, PVN-NPY rats had accumulated 167 ± 11.5% more weight than PVN-contr rats, while LH-NPY rats had accumulated 227 ± 25.7% more weight than LH-contr rats, which was significantly more than the PVN-NPY rats ($p < 0.05$).
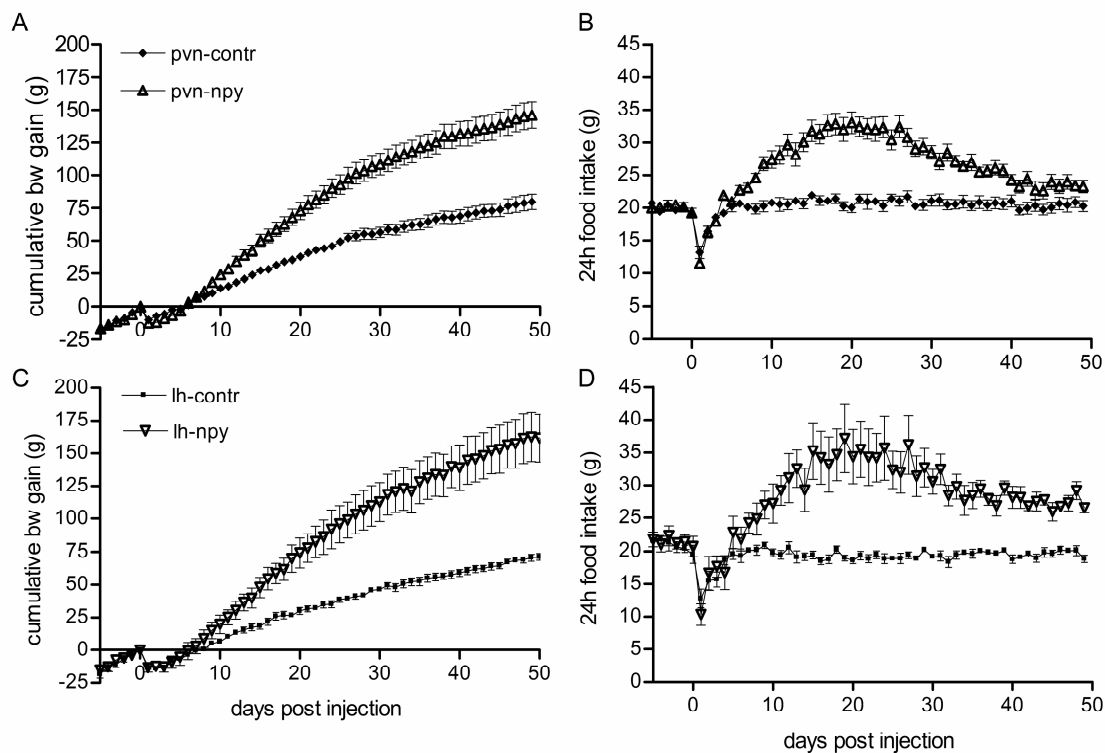
Figure 2: Effects of rAAV-NPY injections in the PVN or LH on cumulative body weight gain (A and C), daily food intake (B and D) compared to rAAV-contr rats.

Daily food intake of both PVN-NPY and LH-NPY rats increased from day 7 after injection until day 15, when it reached a plateau. In PVN-NPY rats, food intake slowly decreased again from day 27 and was similar to controls in the last ten days of the study (Fig. 2C). In LH-NPY rats food intake also decreased from day 27, however, it stabilized at a level that was still significantly increased when compared to LH-contr rats (Fig. 2D), even when food intake was corrected for body weight (day 49: 5.32 ± 0.16 g/100g body weight vs 4.79 ± 0.16 g/100g body weight for LH-NPY vs LH-contr, $p < 0.05$).

Daily water intake of PVN-NPY rats followed the food intake pattern, and was significantly increased between day 11 and day 29 when compared to PVN-contr rats (average of 28.2 ± 1.37 ml per day vs 23.1 ± 1.10 ml per day for LH-NPY vs LH-contr, p<0.05). There were no differences observed in water intake of LH-NPY and LH-contr rats (data not shown).
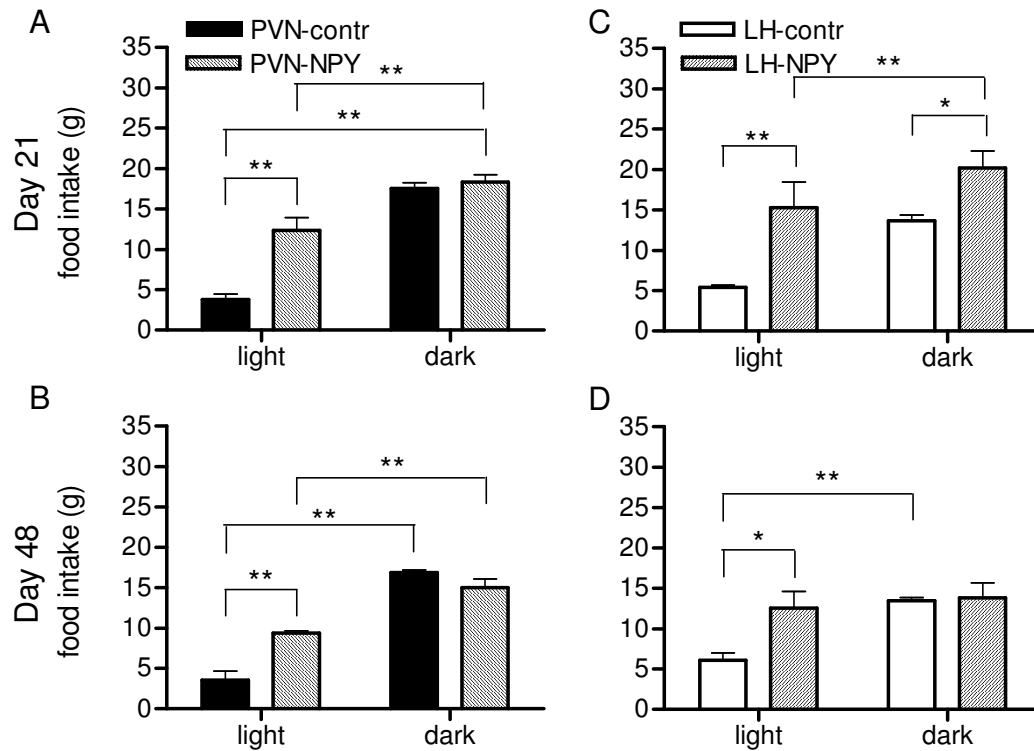
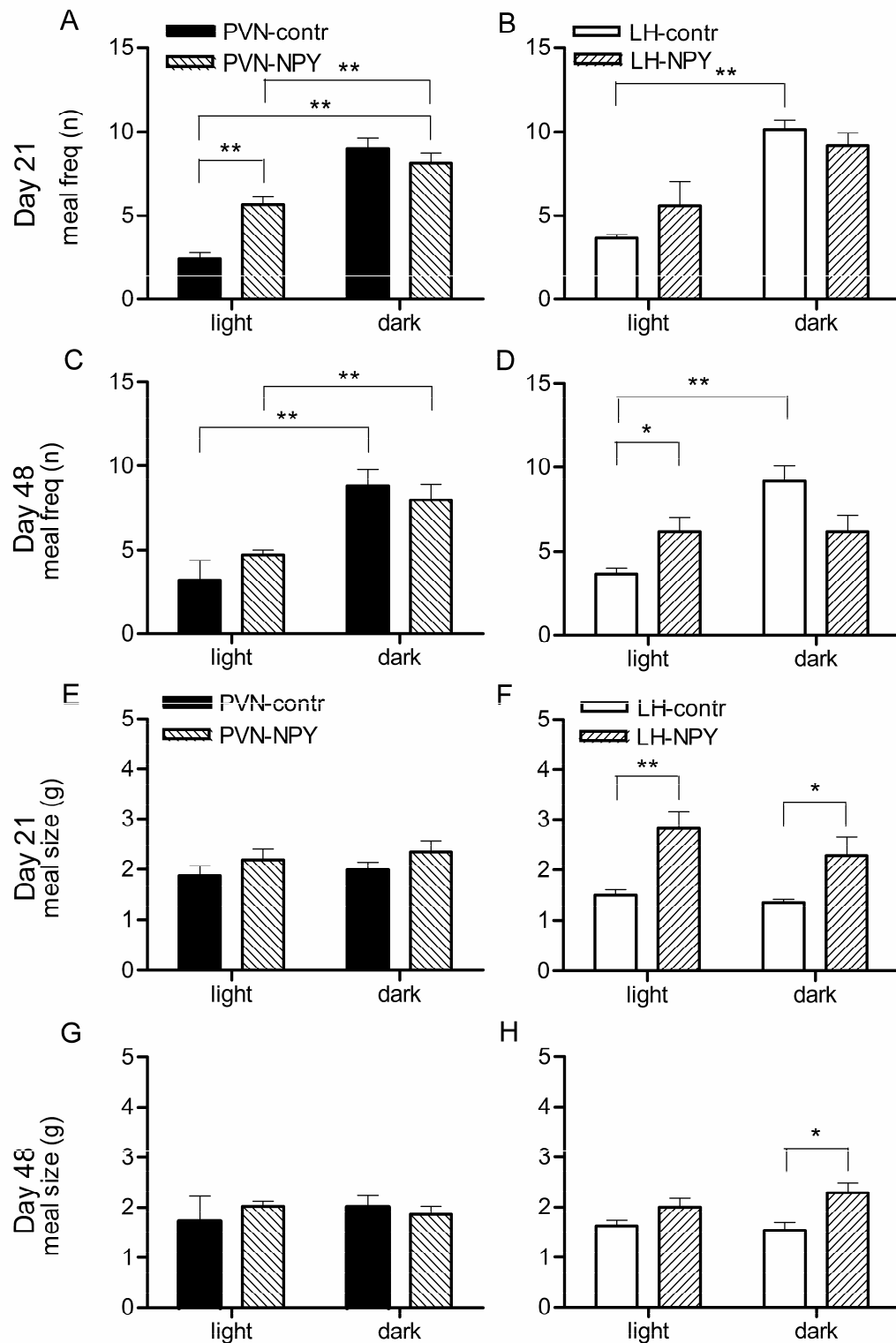

Figure 3: Effects of rAAV-NPY injections in the PVN or LH on dark and light phase food intake on day 21 (A and B) or day 48 (C and D). * p<0.05, ** p<0.01.

AAV-NPY induced effects on meal pattern

As shown above, food intake of rats injected with rAAV-NPY was significantly increased when compared to control rats. To determine whether this increase was due to an increase of meal frequency and/or meal size, meal patterns were analyzed. At day 21, rats that were injected with rAAV-NPY in the PVN showed an increase in light phase food intake, whereas dark phase food intake was similar to control rats (Fig. 3A). Moreover, rats that were injected with rAAV-NPY in the LH showed an increase in both dark phase and light phase food intake (Fig. 3B). At day 48, both PVN-NPY and LH-NPY rats showed a significant increase of food intake only in the light phase (Figs 3C and D).

Both PVN-NPY and LH-NPY rats showed an increased meal frequency in the light phase of day 21 (Figs 4A and B). At day 48, meal frequency of PVN-NPY rats was similar to controls, while LH-NPY rats still showed a significant increase in the light phase (Figs 4C and D). Meal size of rats injected with rAAV-NPY in the PVN was similar to controls both 21

and 48 days after injection (Figs. 4E and G). In contrast, meal size of LH-NPY rats was increased in both the dark and light phase of day 21 and 48 (Figs 4F and H).

Figure 4: Effects of rAAV-NPY injections in the PVN or LH on meal frequency on day 21 (A and B) or day 48 (C and D) and meal size on day 21 (E and F) or day 48 (G and H). * p<0.05, ** p<0.01.

Although PVN-NPY rats showed an increase in meal frequency and total food intake in the light phase, they still ingested significantly more meals and more food in the dark phase as in the light phase, similar to control rats. In LH-PVN rats on the other hand, both the number of meals and total food intake in the light phase was similar to the number of meals and intake in the dark phase at both time points analyzed (Figs 3 and 4).

AAV-NPY-induced effects on body temperature and locomotor activity

To determine whether NPY overexpression in the PVN and LH has differential effects on energy expenditure, we examined body temperature and locomotor activity on day 21 and 48 following injection of the viral particles.

Dark phase body temperature was significantly decreased in both PVN-NPY and LH-NPY rats when compared to controls on both timepoints analyzed (Table 1). In contrast, both groups of rats injected with rAAV-NPY showed a significant increase in body temperature in the light phase of day 21. On day 48 however, no differences were observed in light phase body temperature (Table 1).

We further analyzed the daily pattern of body temperature (Fig 5). Whereas PVN-NPY rats showed a normal circadian temperature rhythm with clear peaks on both days (despite a decrease in dark phase temperature) (Figs 5A and C), LH-NPY rats showed a flattened pattern on both day 21 and 48 (Figs 5B and D).

Locomotor activity of rats injected with rAAV-NPY in the PVN was reduced only in the dark phase of day 21 and 48, whereas activity of LH-NPY rats was, besides in the dark phase of day 21 and 48, also reduced in the light phase of day 21 (Table 1).

Table 1: Effects of rAAV-NPY injections in the PVN or LH on average body temperature and locomotor activity.

		pvn-contr	pvn-npy	lh-contr	lh-npy
Body temp (º Celsius)					
day 21	light	36.92 ± 0.057	37.08 ± 0.024*	36.99 ± 0.041	37.19 ± 0.063*
	dark	37.78 ± 0.039	37.50 ± 0.036**	37.67 ± 0.031	37.47 ± 0.079*
day 48	light	36.89 ± 0.042	36.89 ± 0.042	36.98 ± 0.072	37.03 ± 0.056
	dark	37.64 ± 0.060	37.26 ± 0.065**	37.63 ± 0.054	37.25 ± 0.132*
Activity (% basal)					
day 21	light	94.6 ± 8.7	89.5 ± 7.6	87.7 ± 4.7	63.1 ± 7.8*
	dark	116.9 ± 9.2	72.5 ± 5.7**	85.1 ± 4.5	55.4 ± 9.1*
day 48	light	75.6 ± 11.3	85.5 ± 11.4	87.5 ± 6.9	76.4 ± 13.3
	dark	102.5 ± 11.4	66.5 ± 5.3**	83.2 ± 6.3	48.8 ± 8.5*

Activity data are presented as percentage of values before injection. *p<0.05, ** p<0.01
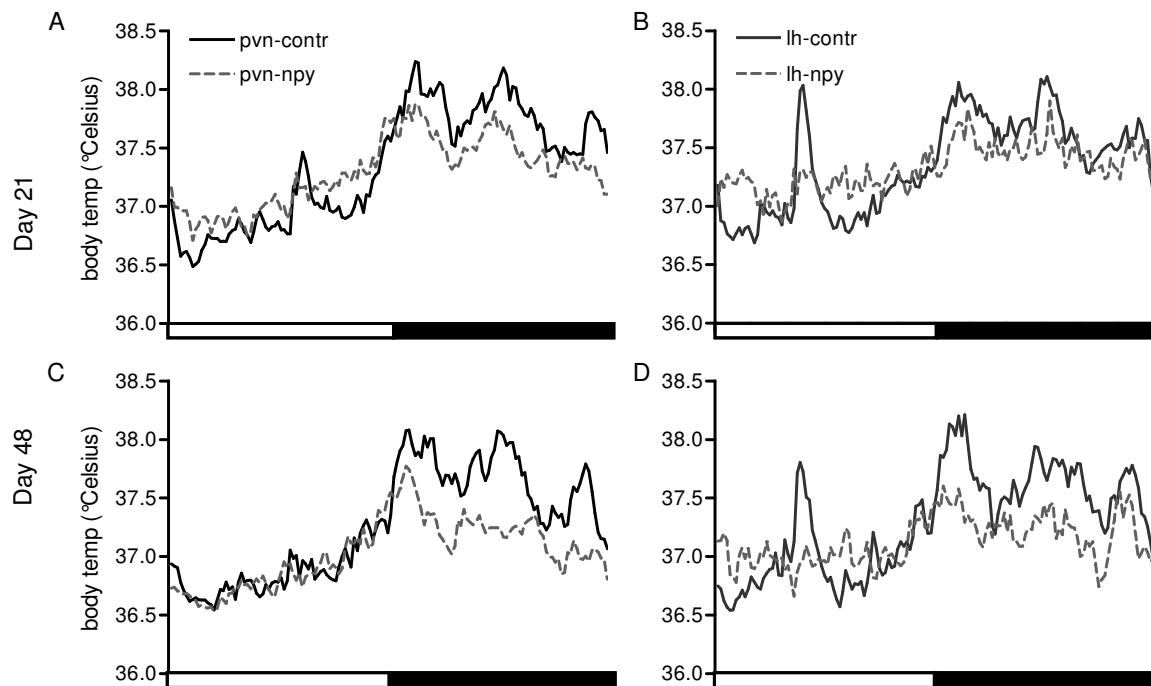
Figure 5: Effects of rAAV-NPY injections in the PVN or LH (data averaged per group) on daily temperature rhythm on day 21 (A and B) or day 48 (C and D).

Table 2: Effects of rAAV-NPY injections in the PVN or LH on endocrine parameters and body composition.

	pvn-contr	pvn-npy	lh-contr	lh-npy
leptin (ng/ml)	4.62 ± 0.50	19.53 ± 2.86**	4.79 ± 0.65	18.86 ± 3.44**
insulin (ng/ml)	4.27 ± 0.67	6.72 ± 0.58*	3.46 ± 0.69	5.99 ± 0.55*
glucose (mmol/l)	5.60 ± 0.11	6.14 ± 0.18*	5.52 ± 0.32	5.73 ± 0.24
cort (μg/dl)	9.70 ± 2.04	20.96 ± 4.62*	3.77 ± 1.13	16.15 ± 8.99
ACTH (pg/ml)	130.81 ± 6.72	311.00 ± 58.61*	104.34 ± 8.50	159.01 ± 40.34
SWAT (%bw)	0.38 ± 0.04	1.15 ± 0.18**	0.46 ± 0.04	1.59 ± 0.20**
AWAT (%bw)	1.98 ± 0.17	4.44 ± 0.45**	1.99 ± 0.09	5.04 ± 0.42**
adrenals (‰bw)	0.09 ± 0.008	0.08 ± 0.055	0.10 ± 0.005	0.09 ± 0.087
thymus (‰bw)	0.91 ± 0.055	0.098 ± 0.088	0.91 ± 0.087	0.79 ± 0.082
pituitary (‰bw)	0.023 ± 0.002	0.018 ± 0.001*	0.024 ± 0.001	0.017 ± 0.001**

SWAT: subcutaneous white adipose tissue, AWAT: abdominal white adipose tissue. * $p<0.05$, ** $p<0.01$

AAV-NPY-induced effects on endocrine parameters and body composition

Table 2 summarizes the effects of NPY overexpression in the PVN and LH on endocrine parameters and body composition.

Subcutaneous and abdominal fat pads and plasma concentrations of leptin and insulin were significantly increased in both PVN-NPY and LH-NPY rats. Whereas plasma glucose levels were normal in rats injected with rAAV-NPY in the LH, rats injected in the PVN displayed increased concentrations of plasma glucose when compared to controls.
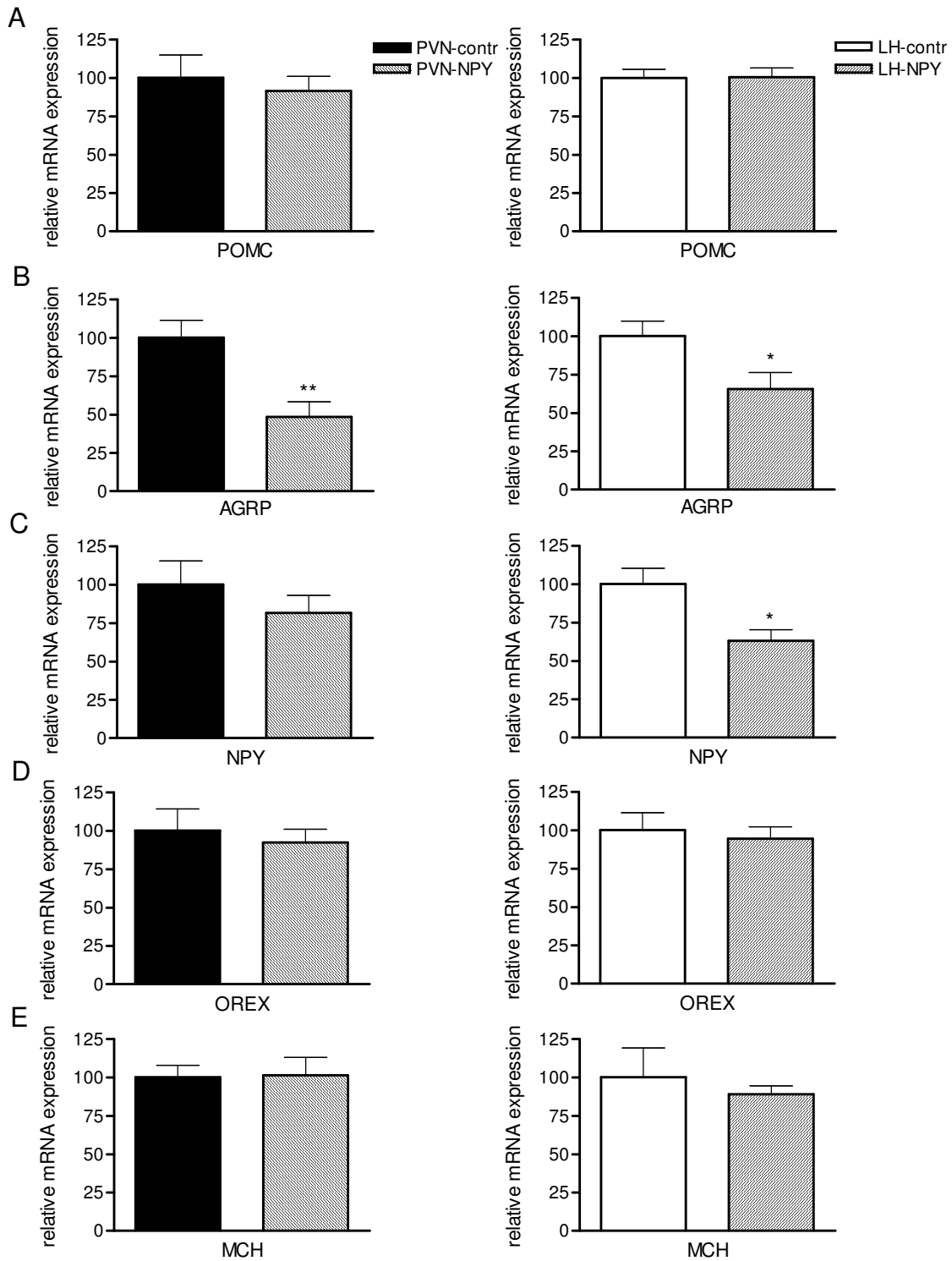
Figure 6: Effects of rAAV-NPY injections in the PVN or LH on mRNA expression of POMC (A), AgRP (B), NPY (C) in the Arc, and orexin (D) and MCH (E) in the LH. * $p < 0.05$, ** $p < 0.01$.

Plasma concentrations of both corticosterone and ACTH were increased in PVN-NPY rats when compared to PVN-contr rats, but not significantly different between LH-NPY and LH-contr rats. No differences were found in the weight of the thymus or adrenal glands, but the weight of the pituitary gland was significantly decreased in both PVN-NPY and LH-NPY rats.

AAV-NPY-induced effects on neuropeptide expression in the Arc and LH

Fifty days after injection of the viral particles, mRNA expression of NPY, AgRP and POMC in the arcuate nucleus, and orexin and MCH was measured by raISH (Fig. 6). When compared to control rats, rats injected with rAAV-NPY in both the PVN and LH showed a similar reduction of AgRP mRNA in the Arc, but no change in POMC mRNA (Figs 6A and B). In LH-NPY, but not PVN-NPY rats, also expression of NPY mRNA was significantly reduced (Fig. 6C). Expression of orexin and MCH in the LH were not altered in rats injected with rAAV-NPY when compared to rats injected with rAAV-contr (Figs 6C and D).

DISCUSSION

Both PVN-NPY rats and LH-NPY rats developed obesity, accompanied by an increase in food intake and concentrations of leptin and insulin, and a decrease in dark phase body temperature and locomotor activity. However, further analysis revealed clear differences in how obesity was induced when NPY was chronically overexpressed in these brain regions. While in PVN-NPY rats food intake was elevated by a specific increase in the number of meals eaten in the light phase, in LH-NPY rats also meal sizes in dark and light phase were increased. Moreover, in LH-NPY rats, but not PVN-NPY rats, circadian rhythmicity with regard to food intake and body temperature was lost. A comparison of the effects observed after injection of AAV-NPY in the PVN and LH is presented in table 3.

NPY overexpression in the PVN or LH resulted in an increase in food intake, which was similar and maximal between day 15 and 27 post injection. Subsequently, PVN-NPY rats reduced their food intake until both food intake and body weight gain was comparable to controls. However, in LH-NPY rats, despite a small decrease, food intake and body weight gain remained elevated when compared to controls.

In PVN-NPY rats, eventually compensatory mechanisms are effective to normalize food intake to control levels, possibly reflecting a new level of body weight or adiposity (Chapter 5). In LH-NPY rats however, food intake remains increased and can not be compensated for completely, despite reduced expression levels of NPY and AgRP mRNA in the Arc. Electrical stimulation of the LH is known to initiate a feeding motor program even in satiated rats (365). In addition, NPY terminals in the LH innervate MCH and orexin neurons (366;367), which project to areas that are important for salivation, arousal and locomotor activity (30;368). NPY signaling in the LH might therefore be more involved in

aspects leading to feeding, which could be less sensitive to compensatory effects such as increased levels of leptin and decreased expression of AgRP and NPY in the Arc. Indeed, although food entrained rats show an increased Fos expression and multiple unit activity in the LH and PVN in anticipation to food, only the food-entrained rhythm of the LH persists after fasting (369;370). This suggests that the LH, rather than the PVN, is involved in aspects prior to the initiation of feeding.

Table 3: Comparison of the effects observed after injection of AAV-NPY in the PVN and LH.

	day 21		day 48	
	PVN	**LH**	**PVN**	**LH**
body weight gain	↑	↑	↔	↑
fat %			↑	↑
food intake	↑	↑	↔	↑
meal size	↔	↑	↔	d↑
meal freq	↑	↔	↔	l↑
water intake	↑	↔	↔	↔
body temp	l↑, d↓	l↑, d↓	d↓	d↓
locomotor act	d↓	↓	d↓	d↓
body temp rhythm	↔	↓	↔	↓
feeding rhythm	↔	↓	↔	↓
plasma glucose			↑	↔
HPA axis			↑	↔

l: light phase, d: dark phase

Analysis of meal patterns revealed that, in the period when daily food intake was maximal, PVN-NPY rats only increased food intake in the light phase, whereas LH-NPY rats ingested more food in both the light and the dark phase when compared to controls. It has been reported that a 4 hour infusion of NPY in the third ventricle increases meal frequency (195). In addition, a single injection of NPY in PVN increases the size of the first meal after injection rather than meal numbers (220;371), whereas a single injection of NPY in the PFA increases food intake due to both size and frequency of meals (227). This is partly consistent with the data described here. We have found that the increase in feeding of PVN-NPY rats was due to an increase in the frequency of normal sized meals, while LH-NPY rats consumed larger meals. Thus, although short-term effects of NPY on food intake may only increase the initiation of meals via signaling in the PVN, effects of long-term overexpression may also involve NPY signaling in the LH, thereby also altering meal size.

Thus far, acute effects of NPY in the PVN on body temperature were contradictory (204;205;223), and effects of NPY in the LH depend on the dose injected (204;205). However, after chronic overexpression of AAV-derived NPY, both rats injected in the PVN and rats injected in the LH show relative hypothermia in the dark phase and (temporal) relative hyperthermia in the light phase when compared to controls. It has been hypothesized

previously that, although NPY signaling in the LH/PFA is important for energy intake, it is not involved in energy expenditure, since body temperature, locomotor activity and respiratory quotient are not altered following an acute injection of NPY in the PFA (204;205;224). Nevertheless, reductions in both dark phase body temperature and locomotor activity were observed in PVN-NPY and LH-NPY rats. In addition, LH-NPY rats also showed a transient reduction in light phase locomotor activity, despite an increased body temperature during this phase of the day. The effects on temperature and activity levels in LH-NPY rats may largely be caused by an increased NPY signaling in the LH, and not by an increase of NPY in the PFA. However, also one rat that had an overexpression of NPY limited to the PFA showed these reductions. Although we can not exclude that this was caused by release of viral-derived NPY to the LH, it is possible that both the PVN and the LH are involved in the effects of NPY on long-term energy expenditure.

Despite the changes in average body temperature, PVN-NPY rats display a normal circadian rhythm, while this is not true for LH-NPY rats. In these rats, although some oscillation is still observed, the overall amplitude of the circadian temperature pattern is markedly reduced. Furthermore, despite the fact that PVN-NPY rats increased only their light phase food intake, they still ingested more food during the dark phase, which is the normal time for a rat to eat. LH-NPY rats on the other hand consume as much food in the light phase as in the dark phase, by decreasing the number of meals consumed in the dark phase and increasing meal frequency in the light phase. This may be explained by the fact that either ICV or LH injections of NPY induce wakefulness (372), probably via orexin neurons. Interestingly, the suprachiasmatic nucleus, which drives the circadian rhythm of feeding activity, is hypothesized to accomplish this via projecions to the orexin and MCH neurons of the LH (373). A continuous stimulation by increased NPY signaling to the orexin neurons and thus the motor circuits involved in feeding behavior would therefore explain the increased anticipation to eat as well as the time spent feeding. Together with the blunted temperature pattern of LH-NPY rats, this provides evidence for a role of NPY signaling in the LH in daily rhythms of both food intake and body temperature.

One could argue that, due to the increase in food intake, also water intake should increase. Indeed, acute injections of NPY in the PVN have been reported to result in an increase in water intake, which is not observed after injections of NPY in the LH (374). In line with this, we observed a small increase in water intake in PVN-NPY rats, whereas LH-NPY rats drank the same amount of water as controls, despite their increase in food intake. It can therefore be concluded that the increased drinking behavior of PVN-NPY rats is not simply a reflection of increased feeding behavior. This is strengthened by the fact that the drinking response is also observed in the absence of food when NPY is injected into the PVN (220).

NPY is known to have a stimulating effect on the HPA axis (203;354) and in turn an increase in HPA axis activity is associated with obesity (355). Therefore, we also investigated the effects of NPY overexpression on the HPA axis. Earlier we have found that AAV-induced NPY overexpression in the PVN did not increase HPA axis activity 23 days after injection (Chapter 4). However, 50 days after injection, we observed an increase in plasma concentrations of ACTH and corticosterone in rats that were injected with AAV-NPY in the PVN. Rats that were injected with AAV-NPY in the LH however did not alter the activity of the HPA axis. This suggests that the effects of NPY on the HPA axis are specifically regulated by the PVN. This is in line with the fact that CRF is produced in neurons of the PVN, but not in the LH (375). Nevertheless, the effects on the HPA axis become evident at a later time-point than the effects on food intake.

In conclusion, these results show that, although chronic NPY administration results in a common obese phenotype, there are region-specific effects of NPY overexpression. Whereas both areas can alter energy expenditure in a similar manner, NPY signaling in the PVN is involved in the initiation of food intake and activation of the HPA axis. Increased NPY signaling in the LH, on the other hand, increases meal frequency and size, and can alter circadian patterns to a status where an animal is constantly ready to eat, thereby equalizing light and dark phase food intake and body temperature. This suggests that the NPY system differentially regulates energy intake and energy expenditure in the hypothalamic nuclei, which together adjust energy balance.