Chapter 5

rAAV-mediated NPY overexpression in the mediodorsal hypothalamus reveals a role for NPY in body weight set-point in adult rats

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

RAAV-MEDIATED NPY OVEREXPRESSION IN THE MEDIODORSAL HYPOTHALAMUS REVEALS A ROLE FOR NPY IN BODY WEIGHT SET-POINT IN ADULT RATS

ABSTRACT

In contrast to strong pharmacological effects of NPY on body weight gain, germline overexpression or deletion of the NPY gene has only modest effects. Using another experimental approach, we bypassed developmental compensations that may be induced with germline NPY overexpression, and injected rAAV-NPY in the mediodorsal hypothalamus. We examined body weight gain and food intake for 50 days in rats that were either pair-fed or fed *ad libitum*.

Rats injected with rAAV-NPY increased food intake for four weeks, where after it normalized again, but had a continuous reduction in activity and body temperature, independent from food intake. We only found a compensatory effect on AgRP mRNA levels, whereas POMC, TRH, CRF, Orexin, MCH and endogenous NPY (in the arcuate nucleus) were unchanged. As soon as a certain body weight was reached following increased NPY signaling, compensatory mechanisms were triggered and food intake was normalized.

Rats that were pair-fed for three weeks and then allowed *ad libitum* food, increased their food intake, which normalized when the rats had reached the same body weight as rats that were *ad libitum* fed the entire study. These data support a role for NPY in body weight set-point regulation.

INTRODUCTION

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the brain. NPY-ergic neurons that are involved in in the regulation of energy balance are mainly expressed in the arcuate nucleus and project to a variety of areas, including the paraventricular (PVN), lateral (LH) and dorsomedial (DMH) nuclei of the hypothalamus and the medial preoptic area (56;177-179). Chronic central administration of NPY results in an obese phenotype, characterized by hyperphagia, increased lipogenesis in liver and adipose tissue and elevated plasma concentrations of leptin, insulin and corticosterone (121;201;203;212;214). Consistently, suppression of endogenous NPY levels by antisense oligonucleotides reduces body weight gain, food intake and insulin secretion (215;218;219;358).

Unexpectedly, NPY transgenic animal models show normal food intake and body weight gain when fed on regular chow (230;231;233). Whether this is due to a developmental change, compensation by counter-regulatory mechanisms or only limited overexpression is not known. Interestingly, also in models where the genes for NPY or one of its receptors are disrupted there are no, or even unexpected phenotypes with regard to body weight and food intake. Deletion of NPY has no effect on food intake and body weight gain, unless animals are fasted for 24 or 48 hours (235;238), suggesting that NPY is more important for situations when food is scarce than for normal feeding behavior. Models where one of the NPY receptors is knocked out ($Y1^{-/-}$, $Y2^{-/-}$ and $Y5^{-/-}$) surprisingly show no effect on or even an increase in body weight and feeding behavior (185;191;246). This suggests that a chronic

absence or overexpression of NPY during development results in compensation by other systems and indicates a remarkable plasticity of the systems involved in energy balance during development. Indeed, it has been shown recently that ablation of arcuate NPY/AgRP neurons decreases food intake in adult mice, but that they can be destroyed without any effect in neonates (155;156). Taken together, results obtained after pharmacological and genetic studies on the function of NPY are contradictory. Compensatory pathways may only be activated after a longer period of changes in the levels of NPY. This is however difficult to test pharmacologically, because it is not feasible to reliably infuse ligands in local brain regions for more than a week.

Recently, it was shown that if NPY is overexpressed at the adult stage in the hypothalamus of mice, obesity is induced (253). These results are comparable to the results after chronic infusion of NPY, and show that if NPY is overexpressed after development, obesity is induced. Apparently, compensatory mechanisms counteract increased NPY signaling during development, but have limited capacity in adulthood.

In order to investigate whether compensation can also take place in the adult rat after a longer period of NPY overexpression, we injected rAAV-NPY in the mediodorsal hypothalamus and examined body weight gain and food intake for 50 days. By carefully monitoring feeding behavior, locomotor activity and body temperature under *ad libitum* and pair-fed conditions we aimed to unravel how NPY induces obesity and to what extent increased food intake contributes to the obese phenotype.

To identify whether the effects of NPY overexpression on body weight and food intake are compensated for, we measured plasma levels of leptin and insulin, and analyzed gene expression levels of neuropeptides in the arcuate nucleus.

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 0700 h). All experimental procedures were approved by the Committee for Animal Experimentation of the University of Utrecht, Utrecht, The Netherlands.

Experiment 1

Rats were anesthetized with 0.1 ml/100 g im 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 (AAV-NPY) (n=9) or rAAV-NSE-empty (AAV-contr) (n=6) was injected bilaterally into the mediodorsal hypothalamus, with coordinates aiming for the PVN (AP: -1.8 mm from bregma, ML: ±0.3 mm from bregma, and DV: -8.0 mm below the skull). The virus was a kind gift of M.J. During (New York). Production of AAV-NPY has been described previously (339;340). 1 µl of virus ($1x10^8$ 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). In this experiment, all rats were provided with *ad libitum* chow.

Experiment 2

In a second experiment, the first experiment was repeated, however, a part of the animals injected with AAV-NPY were pair-fed to the control animals. Pair feeding consisted in providing the rats every day the same amount of chow that was eaten by the control animals. Therefore, the pair-fed animals were injected with the virus two days later than the control group.

In total, 18 animals were injected with AAV-NPY, and 6 animals with AAV-contr in the PVN. Animals injected with AAV-contr and 9 animals injected with AAV-NPY were fed *ad libitum* during the experiment (AAV-contr and AAV-NPY-al). The other 9 animals injected with AAV-NPY were pair-fed to the control group (AAV-NPY-pf). Animals in experiment 2 had a slightly increased body weight at the moment of injection than animals used in experiment 1.

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. Locomotor activity and body temperature were measured via the transmitters that send digitized data via radio frequency signals to a nearby receiver. The data were automatically recorded every ten minutes, and averaged per hour using DSI software (DSI, St Paul, MN).

Collection of blood and tissues

At day 50 after infection, rats were decapitated, 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 and epididymal white adipose tissue (WAT) was isolated and weighed.

Verification of injection sites

16 μm coronal sections of the hypothalamus were sliced using a cryostat (Leica, Rijswijk, The Netherlands), thaw-mounted onto RNAse free Superfrost slides (Menzel, Germany) and stored at -80 °C. Viral infection was localized by in situ hybridization (ISH) with a digoxigenin (DIG)-labeled woodchuck post-transcriptional regulatory element (WPRE) probe. The WPRE sequence is part of the expression cassette of all vectors used here. ISH procedure has previously been described (341). Results from rats with incorrect injections were excluded from the analysis.

Quantitative in situ hybridization

16 μm coronal sections were used for raISH. 33P-labeled antisense RNA probes were made using AgRP (396 bp mouse (343)), NPY (286 bp rat (344)), POMC (350 bp rat (343)), CRF (770 bp rat), TRH, MCH (500 bp mouse (359)) and prepro-orexin (542 bp rat, AF041241) cDNA fragments. raISH procedure has previously been described (345). Expression of AgRP, NPY and POMC was analyzed in the arcuate nucleus, expression of CRF and TRH was analyzed in the PVN and expression of MCH and prepro-orexin was analyzed in the LH using Image J software (National Institutes of Health, Bethesda, Maryland, USA). Expression of NPY was also measured in the mediodorsal hypothalamus to confirm viral-mediated overexpression.

Plasma analysis

Plasma leptin and insulin were analyzed in duplicate using radioimmunoassay kits, (Linco Research, St Charles Missouri, USA).

Statistical analysis

Data are presented as group means ± SEM. Differences in body weight, food intake, body temperature and locomotor activity were assessed using repeated measure analysis. When significant overall interactions were found, post hoc analyses were performed with T-tests or one-way ANOVA. Differences between fat weight, leptin, insulin and gene expression levels were analyzed by T-tests or one-way ANOVA, followed by Tukey's post-hoc test. Differences were being considered significant at $p<0.05$.

RESULTS

Localization of rAAV in the brain

In order to overexpress NPY in the mediodorsal hypothalamus, we bilaterally injected AAV-NPY or AAV-contr with coordinates aimed for the PVN in the hypothalamus of rats. Correct injection was verified by WPRE mRNA expression (Figs 1A and B), and animals with incorrect injections were excluded from analysis (in total, in experiment 1 three rats from AAV-NPY were excluded, and in experiment 2 one rat from AAV-contr, two rats from AAV-NPY-al, and three rats from AAV-NPY-pf were excluded). Viral-induced NPY expression in the mediodorsal hypothalamus at fifty days post injection was confirmed by RA-ISH (Figs 1C and D). Viral-induced NPY mRNA expression in the mediodorsal hypothalamus of AAV-NPY injected rats was 167.6 ± 12.42 % of arcuate nucleus expression of controls (data not shown).

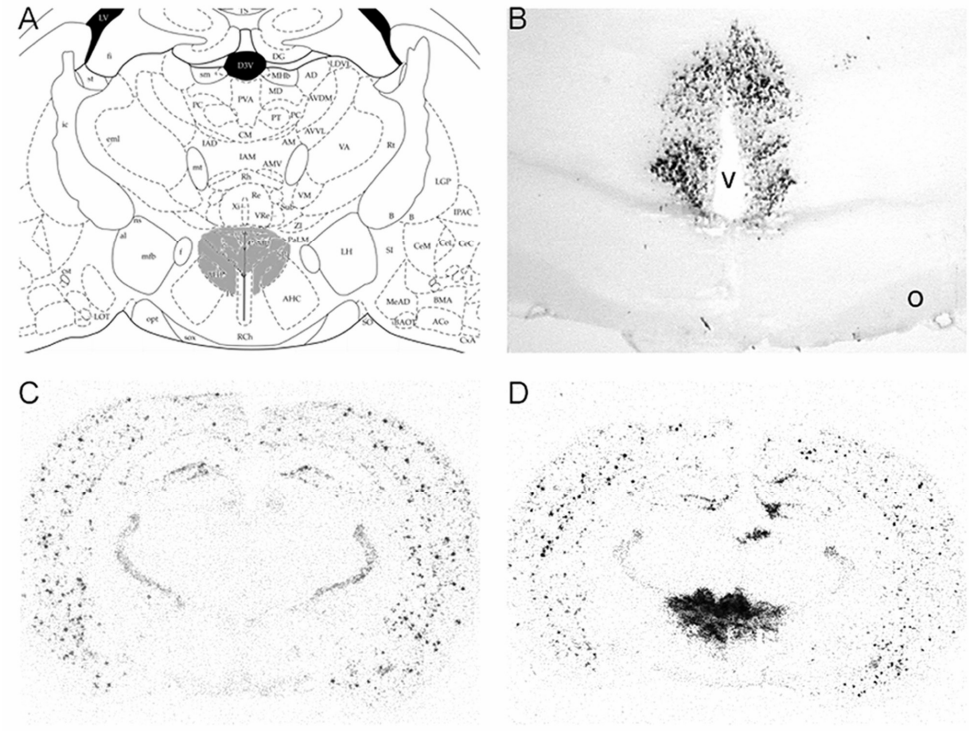

Figure 1: A. Schematic example of the injection area (indicated in grey) of the viral particles; B. Example of WPRE expression in the mediodorsal hypothalamus, as assessed by *in situ* hybridization (o = optic tract, v = third ventricle), adapted from Paxinos and Watson (321); C. Example of NPY mRNA expression in the mediodorsal hypothalamus of a rat injected with AAV-contr; D. Example of NPY mRNA expression in the mediodorsal hypothalamus of a rat injected with AAV-NPY.

Overexpression of NPY in the mediodorsal hypothalamus temporary increases food intake and body weight

Following injection of the rAAV particles in the mediodorsal hypothalamus, all animals showed a similar drop in body weight and food intake. Body weight and food

intake were back to pre-surgery levels within one week after injection. Food intake was increased in rats injected with AAV-NPY from day 7. Daily food intake increased until day 15, when it was 1.6 times higher than control animals. Food intake remained stable between day 15 and day 27, after which it slowly decreased until it was comparable to AAV-contr injected animals from day 40 post injection until the end of the study (Fig. 2A).

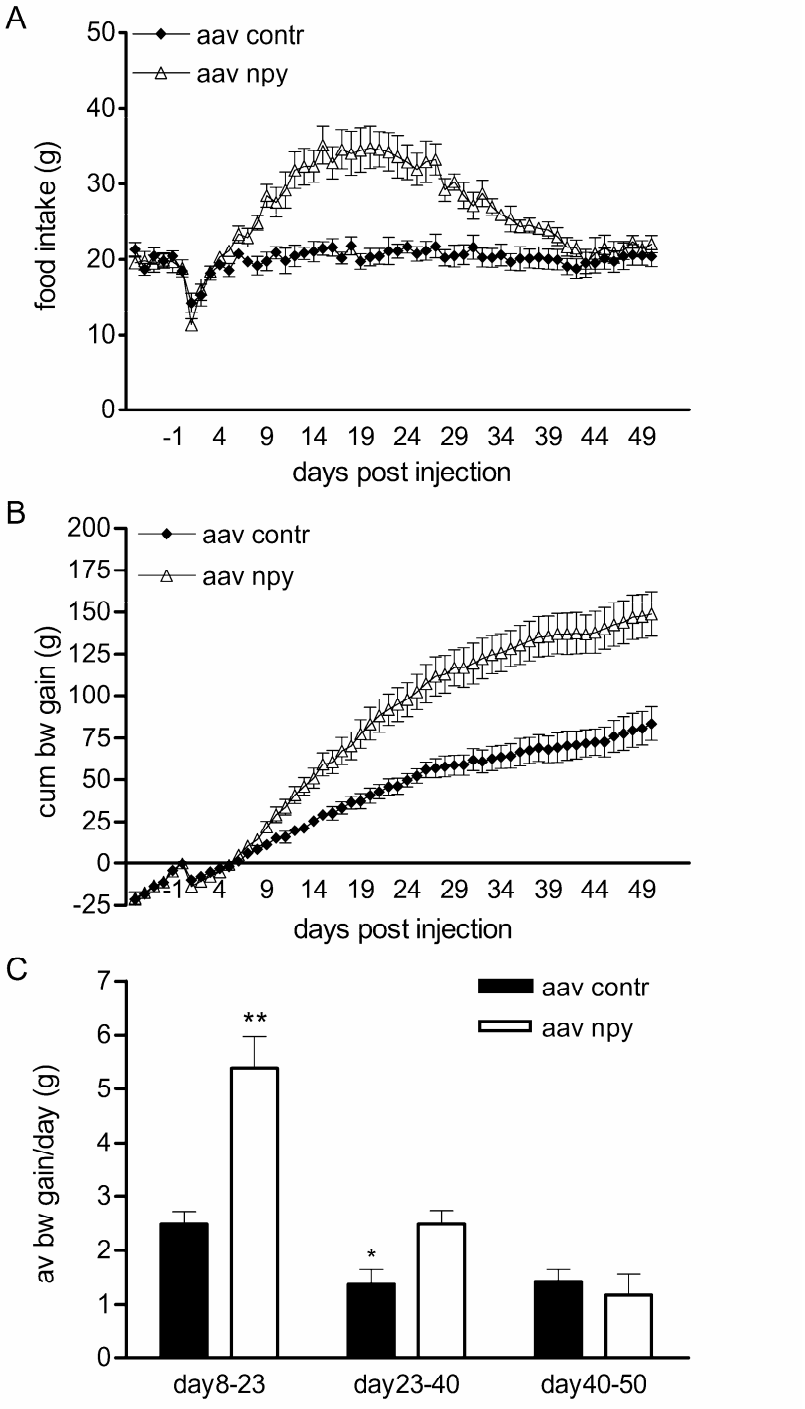

Figure 2: Effects of rAAV-NPY injection in the mediodorsal hypothalamus on daily food intake (A), cumulative body weight gain (B) and average body weight gain/day; (C). * $p<0.05$, ** $p<0.01$.

From day 9 after injection, rats injected with AAV-NPY showed an increase in body weight gain, which resulted in an accumulated body weight gain of 178% more than controls at the end of the study (Fig. 2B). Differences in body weight gain of rats injected with AAV-NPY and AAV-contr were highest between day 8 and day 23 (average of 5.38 ± 0.60 vs 2.49 ± 0.24 g/day for AAV-NPY vs AAV-contr, $p<0.01$), slowly decreased between day 23 and 40 (average of 2.49 ± 0.26 vs 1.36 ± 0.29 g/day for AAV-NPY vs AAV-contr, $p<0.05$) and was not significant anymore in the last ten days of the study (average of 1.17 ± 0.39 vs 1.42 ± 0.23 g/day for AAV-NPY vs AAV-contr) (Fig. 2C).

Table 1: Effects of overexpression of NPY in the mediodorsal hypothalamus on body weight gain, relative WAT, and plasma concentrations of leptin and insulin, 50 days post injection.

	Bw gain (g)	WAT (%bw)	Leptin (ng/ml)	Insulin (ng/ml)
aav-contr	83.5 ± 9.99	3.17 ± 0.35	4.76 ± 0.67	3.10 ± 0.67
aav-npy	148.9 ± 13.06**	5.64 ± 0.39**	20.63 ± 4.61**	5.84 ± 0.75#

p=0.08 vs contr, ** p<0.01 vs contr

Compensatory effects of NPY overexpression

Since there is still an obvious overexpression of NPY in the mediodorsal hypothalamus at day 50, which is comparable to overexpression at day 23 (145.0 ±10.1% of arcuate nucleus expression of controls, B. Tiesjema, unpublished observations), the reduction of effects on food intake and weight gain are not due to decreased viral-mediated overexpression of NPY.

The increased body weight gain and WAT stores were accompanied by increased plasma leptin and insulin levels (table 1). Leptin levels were significantly elevated in rats injected with AAV-NPY (20.63 ± 4.61 vs 4.76 ± 0.67 ng/ml for AAV-NPY vs AAV-contr, $p<0.01$). There was also a tendency towards hyperinsulinemia in rats injected with AAV-NPY (5.84 ± 0.75 vs 3.10 ± 0.67 ng/ml for AAV-NPY vs AAV-contr, $p=0.08$).

Next we measured expression of well known orexigenic and anorexigenic neuropeptides in the hypothalamus in order to determine to what extent these systems adapt to NPY overexpression and increased leptin signaling. We have shown earlier that 23 days after AAV-NPY injections in the PVN, AgRP mRNA expression is reduced to 40% when compared to controls, while NPY and POMC expression is similar to controls (Chapter 4). The mRNA expression of NPY, AgRP and POMC in the arcuate nucleus was measured with raISH 50 days after injection of the viral particles. Although there was no significant difference in mRNA expression of NPY or POMC in the arcuate nucleus of rats injected with AAV-NPY when compared to control animals, AgRP expression was significantly reduced to 50% of control values (Fig. 3a). Neuropeptide expression in the PVN (CRF and TRH) and in the lateral hypothalamus (MCH and orexin) were not altered in animals injected with AAV-NPY (Figs 3B and C).

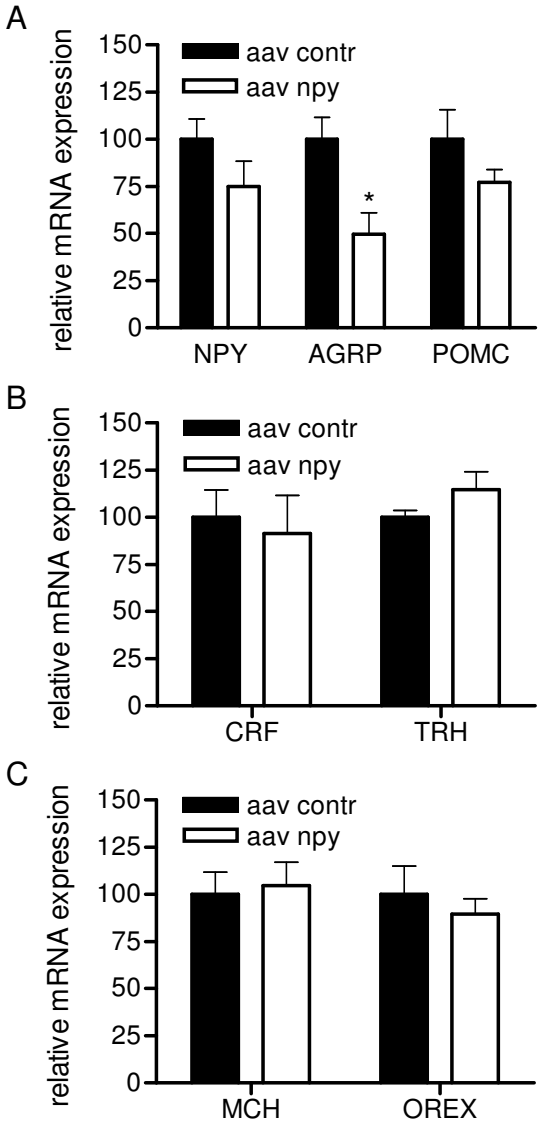

Figure 3: Effects of rAAV-NPY injection in the mediodorsal hypothalamus on mRNA expression of NPY, AgRP and POMC in the arcuate nucleus (A), CRF and TRH expression in the PVN (B) and MCH and orexin expression in the LH (C). * $p<0.05$.

Food independent decrease of body temperature and locomotor activity by NPY overexpression in the mediodorsal hypoyhalamus

To determine to what extent the effects of NPY overexpression on body weight gain and energy expenditure were dependent on the increase in food intake, a pair-fed study was performed (experiment 2). As shown in figure 2, also a new group of rats that were injected with AAV-NPY and fed *ad libitum* increased their food intake until day 18, whereafter it was stable for about 10 days before it normalized (Fig. 4A). Body weight gain increased until day

27, thereafter it was not significantly different anymore from controls (Figs 4B and C). This is consistent with the results from experiment 1.

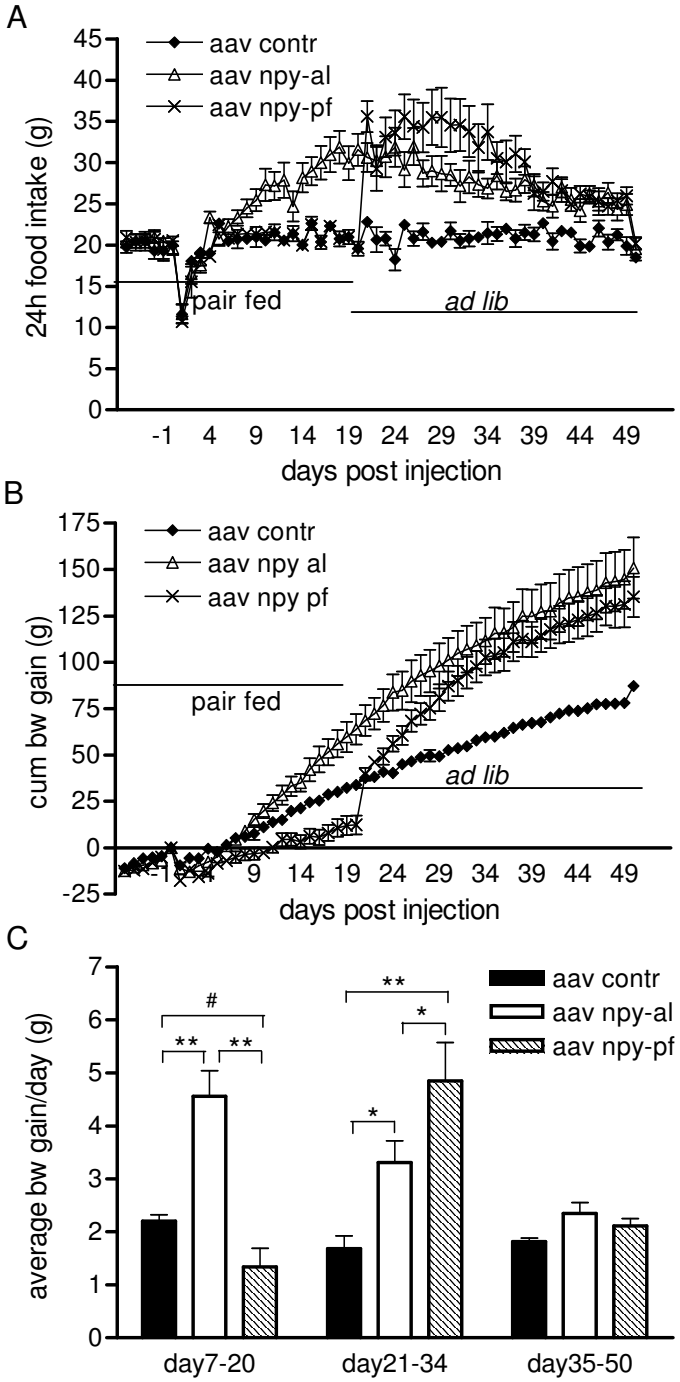

Figure 4: Effects of rAAV-NPY injection in the mediodorsal hypothalamus on daily food intake (A), cumulative body weight gain (B) and average body weight gain/day (C) in rats that are either pair-fed or fed *ad libitum*. # p=0.07, * p<0.05, ** p<0.01.

AAV-NPY rats that were pair-fed to the AAV-contr rats, showed a decreased body weight gain when compared to controls (Figs 4B and C), which resulted in a cumulative

body weight gain that was significantly lower than AAV-contr rats twenty days after injection (12.28 ± 5.22 vs 33.9 ± 2.12 g for AAV-NPY-pf vs AAV-contr rats, $p<0.01$). The rats that were pair-fed consumed all their food in the first half of the dark period (data not shown).

Effects of AAV-NPY on body temperature are shown in Figures 5A and B. Although light phase body temperature tended to be increased in AAV-NPY injected animals, this was not significant (Fig. 5A). However, in the dark phase, body temperature was significantly reduced in AAV-NPY injected animals, starting two weeks after injection (Fig. 5B). Body temperature was equally decreased in *ad libitum* fed and pair-fed animals (Note that the AAV-NPY-pf group is pair-fed until day 21, where after it is fed *ad libitum*). Dark phase body temperature remained significantly reduced during the entire experiment.

Light phase locomotor activity of rats injected with AAV-NPY was not significantly different from animals injected with AAV-contr (Fig. 5C). Dark phase activity was reduced in both AAV-NPY-al and AAV-NPY-pf animals on day 6, and remained significantly reduced throughout the rest of the experiment (Fig. 5D).

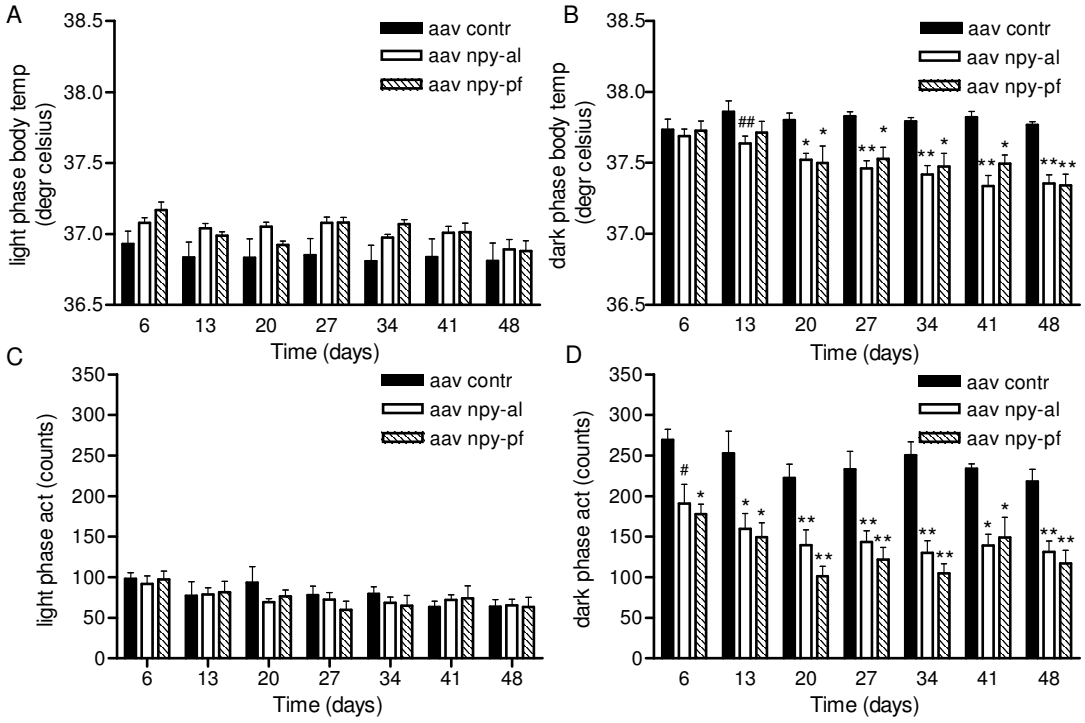

Figure 5: Effects of rAAV-NPY injection in the mediodorsal hypothalamus on body temperature in the light (A) and dark phase (B), and locomotor activity in the light (C) and dark phase (D). #p=0.06, ## p=0.07, * $p<0.05$, ** $p<0.01$.

Involvement of downstream pathways

To determine whether the normalization of food intake observed in ad libitum fed AAV-NPY injected rats was caused by adaptations downstream of viral-induced NPY

signaling, we allowed the pair-fed animals to eat *ad libitum* from day 21 until the end of the experiment. We reasoned that, if adaptations downstream of NPY were responsible for the normalization of food intake, animals that were pair-fed for the first three weeks of the experiment would follow the food intake curve of the AAV-NPY-al rats from the moment they were given access to *ad libitum* food. Therefore, they should show hyperphagia (to a similar amount of AAV-NPY-al rats) for only one week, and normalize their food intake at the same time of AAV-NPY-al rats, irrespective of their body weight.

Food intake of AAV-NPY-pf animals immediately increased when they were fed *ad libitum*, and food intake remained elevated and stable until day 34 after injection, after which food intake returned to control values (Fig. 4A). Cumulative food intake from day 21-34 of AAV-NPY-pf rats was increased when compared to AAV-NPY-al rats (475.2 ± 39.0 g vs 388.5 ± 16.5 g, $p<0.05$).

In line with this, directly after being fed *ad libitum*, AAV-NPY-pf rats started to increase their body weight gain. Already twenty-four hours after their first day with *ad libitum* food, cumulative body weight gain of AAV-NPY-pf rats was similar to that of control rats (day 21: 39.6 ± 3.38 vs 37.7 ± 1.77 g for AAV-NPY-pf vs AAV-contr rats), and as of 4 days after *ad libitum* food their cumulative body weight gain was significantly higher than controls (day 24: 60.6 ± 5.12 vs 45 ± 2.33 g for AAV-NPY-pf vs AAV-contr rats, $p<0.05$). Between day 21 and 34 body weight gain per day was also increased when compared to AAV-NPY-al animals ($p<0.05$) (Fig. 4C). From day 34 post injection, body weight gain of the AAV-NPY-pf rats slowed down again, and average body weight gain per day was not different anymore from controls and AAV-NPY-al rats.

Fifty days after injection of the viral particles, body weight gain and fat percentage of AAV-NPY-pf animals was similar to that of AAV-NPY-al animals, and significantly increased when compared to control animals (Table 2). Furthermore, plasma concentrations of leptin and insulin were also elevated in comparison with control animals, but not different from AAV-NPY-al animals (Table 2).

Table 2: Effects of overexpression of NPY in the mediodorsal hypothalamus on body weight gain, relative WAT, and plasma concentrations of leptin and insulin in pair-fed and *ad libitum* fed rats, 50 days post injection.

	Bw gain (g)	WAT (%bw)	Leptin (ng/ml)	Insulin (ng/ml)
aav-contr	87.2 ± 1.38	2.18 ± 0.21	4.44 ± 0.85	4.44 ± 0.66
aav-npy-al	150.8 ± 16.45*	5.40 ± 0.53**	18.58 ± 3.86*	7.86 ± 0.51**
aav-npy-pf	135.3 ± 10.91*	5.18 ± 0.57**	17.81 ± 2.62*	7.34 ± 0.89*

* $p<0.05$ vs contr, ** $p<0.01$ vs contr

DISCUSSION

The results described in this study show that long-term overexpression of NPY in the mediodorsal hypothalamus results in a transient increase in food intake and body weight

gain, and a continuous reduction in dark phase locomotor activity and body temperature. When permitted to eat *ad libitum*, food-restricted AAV-NPY injected animals adjust their weight and fat mass to a similar level as non-restricted rats. This implies that increased NPY signaling in the mediodorsal hypothalamus alters body weight set-point. Furthermore, the decline in body temperature and locomotor activity was also observed in pair-fed rats, which indicates that the effects of NPY on energy expenditure are independent from food intake.

Approximately four weeks after virus injection, food intake of AAV-NPY injected rats that were fed *ad libitum* decreased, until it was comparable to intake of control rats. At the same time, body weight gain returned to normal values. This reduction of effects on food intake and weight gain was not due to a decreased amount of viral-derived NPY expression, since NPY mRNA levels in the mediodorsal hypothalamus on day 23 and day 50 were similar. Furthermore, effects on dark phase body temperature and locomotor activity were continuous, also indicating that the extent of viral-mediated NPY overexpression at the end of the experiment was still high enough to elicit effects.

The normalization of food intake and body weight gain in the last phase of our study suggests that counter-regulatory mechanisms are triggered when NPY is overexpressed during the adult stage. Other studies suggest that adaptations to changes in the NPY system can only occur during development or in early stages of life, and that the plasticity of the systems involved in energy balance declines with age (155;156). Nevertheless, the normalization of food intake and body weight gain weeks after the injection of AAV-NPY, as observed in our study, suggests that compensatory pathways are also activated in adult animals, however, it takes some time before adaptation begins. This explains why compensatory effects are not observed after 1 week administration of NPY, which is simply too short to initiate the counter-regulatory mechanisms. Although it is clear that adaptations occur after a chronic situation of imbalance in the NPY system, the mechanisms behind these adaptations are poorly understood.

The adiposity signals leptin and insulin are well known regulators of appetite. When body fat mass increases, plasma levels of leptin and insulin rise (8;9). This results in stimulation of POMC neuronal activity and inhibition of NPY/AgRP neurons in the arcuate nucleus, and thus a decrease in food intake (17). A logical explanation for the normalization of food intake as described above would therefore be that, due to the increased fat mass, plasma concentrations of leptin and insulin rise in AAV-NPY injected rats. Subsequently, this would alter the gene expression patterns of neuropeptides in the arcuate nucleus. Indeed, fat mass and plasma levels of leptin and insulin of AAV-NPY injected rats are elevated at day 50, and although POMC and NPY mRNA expression in the arcuate nucleus was not significantly altered, AgRP expression was reduced. Since AgRP mRNA levels were similarly reduced at 23 and at 50 days of NPY overexpression, compensation via reduced

levels of AgRP mRNA can not explain why food intake was lower at 50 days. However, we cannot exclude that the increased levels of leptin affect other neural circuits that are responsible for the normalization of food intake.

An alternative explanation for the normalized food intake at the end of the experiment is that the increased NPY signaling alters body weight setpoint. The theory that a peptide can adjust the degree of food intake to the current level of body adiposity was first described by Powley and Keesey (360). They hypothesized that an adiposity signal only alters food intake for the period that is needed to achieve the adiposity status that is characteristic for the specific signal, regardless of the initial body weight of the animal (360). Examples of such signals are insulin and MTII (a melanocortin agonist), which only reduce food intake or body weight gain to the extent that is needed to reach a new defended degree of adiposity (361;362). Therefore, these ligands are more effective in animals with a normal weight than in animals that are weight reduced by food restriction (361;362). Since AAV-NPY injected animals that were pair-fed to controls for three weeks before they were allowed *ad libitum* food, increased their food intake until they reached the same body weight as AAV-NPY-al rats, we favor the hypothesis that increased NPY expression increased their set-point to a certain amount of body weight or adiposity. When this set-point is achieved, sensitivity for NPY decreases and food intake normalizes. Since the defence of a certain body weight set-point is also observed in humans (363), it is possible that in some subjects obesity is maintained despite a normal pattern of food intake, by a chronically altered NPY signaling.

Even though food intake of AAV-NPY injected rats normalizes over time, body temperature and locomotor activity remain decreased, indicating that not all effects caused by the high levels of NPY in the hypothalamus are compensated for. Although data on locomotor activity or body temperature in NPY transgenic animals are not reported, $NPY^{-/-}$ animals show no change in these parameters (237;244), suggesting that, if alterations in temperature and activity occur due to absence of NPY during development, they are masked by the contribution of counter-regulatory systems. Since animals with an altered NPY signaling during development have a normal body weight, it appears that altered NPY signaling in prenatal animals does not affect body weight set-point in a similar manner as in adults. Therefore, it is likely that compensatory mechanisms during development differ from the mechanisms in the adult rat. However, it is possible that the secondary mechanisms responsible for the adaptation to either too high or absent NPY levels in the brain are similar.

Although it is reported that one week of ICV administration of NPY does not alter body weight gain when increased food intake is prevented (121;203), AAV-NPY injected rats that were pair-fed to controls showed a lower body weight gain when compared to AAV-contr rats. AAV-NPY-pf rats ingested all their food in the first half of the dark period. Since

NPY overexpression specifically stimulates light phase food intake (Chapter 4), the absence of food in the light phase might be perceived as starvation. Although dark phase central body temperature was decreased in the pair-fed rats, as assessed using telemetry probes, it is possible that the stressful condition of the pair-fed rats increases heat loss via the extremities, which would not be detected by the telemetry probes. In addition, we can not exclude that in pair-fed rats calories are not as efficiently taken up as in control animals, since stress inhibits gastric motility and increases colonic motor function and defecation (364).

In summary, this study provides evidence that NPY overexpression in the mediodorsal hypothalamus of adult rats plays a role in regulation of body weight setpoint. This causes a transient increase in food intake and body weight gain, but an unremitting (food independent) reduction of energy expenditure of rats injected with AAV-NPY. The results support the data that, also in adult rats, the systems involved in the regulation of energy balance show enough plasticity to compensate for the effects of high NPY signaling on feeding behavior.

ACKNOWLEDGEMENTS

This study was supported by NWO ZonMW 903-039-193