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Activity of the hypothalamic neuropeptide Y increases in adult and decreases in old rats

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Middle-aged obesity and aging anorexia with muscle loss (sarcopenia) of old people present public health burden. These alterations may appear both in humans and rodents suggesting the role for regulatory alterations. Previously, we demonstrated that biphasic changes in the weight-reducing (catabolic) effects of neuropeptides of the hypothalamus–adipose tissue axis (e.g. leptin) may contribute to both trends. With regard to the anabolic effects of the hypothalamic neuropeptide Y (NPY) inhibited by leptin, we hypothesized non-linear age-related changes with shifts in the opposite directions. We investigated the orexigenic and hypometabolic effects of intracerebroventricularly administered NPY (hyperphagia induced by NPY injection or changes in food intake, body weight, heart rate, body temperature, locomotor activity during a 7-day NPY infusion), the immunoreactivity and gene expression of NPY in the hypothalamic arcuate nucleus of male Wistar rats of five age groups from young to old. The orexigenic/hypometabolic efficacy and the immunoreactivity of NPY increased in middle-aged animals preceding the peak of adiposity observed in aging rats, then decreased preceding anorexia and weight loss in old rats. These shifts may contribute to the development of both age-related obesity and aging anorexia, sarcopenia, and should be considered in future drug development targeting the NPY system.

Keywords Obesity, Aging anorexia, Metabolism

Two major trends are observed in the long-term regulation of energy balance: age-related obesity followed by aging anorexia leading to weight loss at old age. From adulthood the body fat rises continuously reaching its peak between 55 and 70, followed by a decline[1,2]. This peak appears in the 50s for men and in the 60s for women[3]. Before menopause estrogens protect against adiposity through suppression of appetite and increase in energy expenditure. In addition, estrogens favor lipid accumulation subcutaneously and inhibit it in the visceral region. Decline in estrogens during menopause leads to a shift in adipose tissue deposition favoring the visceral localization and increased metabolic risk reminiscent to that seen in men[4]. Fat distribution changes dramatically throughout life: visceral and intramuscular fat increases with aging, while subcutaneous fat declines in both sexes[5]. In contrast, muscle mass decreases from 40 to 50 years of age accompanied by loss of muscle strength which leads to aging sarcopenia[2,6]. Women are in general weaker than men and have smaller muscles, but aging affects women in a similar way[7]. Both trends shorten health span and life span presenting a growing health burden. In addition to social, psychological factors and diseases, intrinsic regulatory changes may contribute to these processes since they are similar in humans and other mammals, including laboratory rodents[8,9]. Age-related shifts in the activities of hypothalamic neurohumoral systems may play an important role together with alterations in peripheral hormones, neural networks and interactions of peripheral with central circuits[9]. The neuropeptides of the hypothalamus–adipose tissue axis play a pivotal role in energy homoeostasis controlling the nutritional status and energy storage. Our earlier studies in male Wistar rats demonstrated non-linear age-related changes in the central catabolic (appetite reducing and hypermetabolic actions leading to weight loss) effects of leptin, the main adiposity signal from fat tissue and in those of its hypothalamic target, the melanocortin system: a transient decline in their catabolic efficacy preceding middle-aged weight gain, which was followed by an enhancement preceding weight loss in older age groups[10–15]. These shifts may contribute to the development

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of both middle-aged obesity and aging anorexia/sarcopenia. In contrast, female Wistar rats do not show age-related obesity rather maintain their stable low body weight throughout life[16]. Female rats do not experience human-like menopause, their reproductive senescence is characterized by moderate to high estradiol levels[17].

Leptin inhibits neurons in the hypothalamic arcuate nucleus (ARC) producing neuropeptide Y (NPY), a major anabolic (appetite inducing action with decreased energy expenditure leading to weight gain) hypothalamic neuropeptide[9]. Starvation upregulates NPY expression leading to energy-conservation: hyperphagia and hypometabolism acting on hypothalamic second order neurons[18]. These effects are mediated mainly via its Y1 and Y5 receptors[19,20]. Several studies suggest a critical role of NPY for the beneficial anti-aging effects of caloric restriction[21]. Additionally, NPY decreases processes triggered by sympathetic activity: nonshivering thermogenesis in brown adipose tissue and lipolysis in white adipose tissue. They are associated with enhanced adipogenesis shifting fuel utilization to carbohydrates to preserve fat reserve[18,22]. Accordingly, a chronic central NPY infusion was shown to induce increased adiposity in rats by hyperphagia and reduced thermogenesis[23].

Compared with young rodent or human groups, the level and the hyperphagic effect of central NPY decrease at late old age, which contributes to aging anorexia[24]. These data suggest a linear age-related decline. However, to date, no analysis involving more than three age groups or focusing on the hypometabolic effects of NPY was published. We hypothesize that age-related dynamics in NPY activity is non-linear, shifting in the opposite directions than those seen in catabolic systems: first it may increase promoting age-related obesity and only later decreases leading to aging anorexia and weight/muscle loss. To test this hypothesis an appropriate animal model involving more than three age groups is needed. Based on the assessment of body composition, muscle strength and food intake (FI) we established our model applying male Wistar rats. We investigated the hyperphagic and hypometabolic effects of intracerebroventricularly (ICV) administered NPY in vivo and the endogenous NPY activity at mRNA and peptide levels in the ARC of five age groups from young to old (from 3 to 24 months). Dynamics of these age-related changes were then compared with those of the in vivo results.

Results

In vivo results: increased anabolic efficacy of NPY precedes the peak of middle-aged weight gain, a decreased efficacy precedes weight loss in old rats

In our male Wistar rats, development in body weight (BW) and adiposity (indicated by adiposity index) show a continuous age-related rise until 18 months of age. A rapid growth period until 6 months of age is followed by a moderate rise reaching the peak at 18 months (Fig. 1a, b). Pronounced BW decline is observed in the oldest 24-month-old animals, which is combined with a significant loss of both fat and muscle mass (Fig. 1a–c). In order to assess muscle loss we introduced a novel muscle index based on weight of muscle samples expressed as % of actual BW. Interestingly, this muscle index was also significantly reduced in the 18-month-old rats demonstrating a remarkable age-related muscle loss already at the age of the highest BW and body fat (Fig. 1c cp. panels a and b). Their high BW itself does not explain this reduction of muscle index, since the absolute wet weights of their dissected muscles were also significantly lower than in the 12-month-old (middle-aged) rats with similar BW (1.47 ± 0.04 g vs. 1.81 ± 0.10 g, $p = 0.012$, one-way ANOVA with Tukey's post hoc test). In summary, fat along with muscle mass increases until the age of 12 months, then the rats accumulate excess fat during the following 6 months, while starting to lose muscle. Nevertheless, the forelimb grip strength was maintained in the 18-month-old animals. Only the oldest 24-month-old rats exhibited reduced grip strength (Fig. 1d). This reduction in muscle strength and mass indicates aging sarcopenia. At this age their spontaneous daily FI also decreases indicating aging anorexia (Fig. 1e).

We investigated the effect of NPY on FI upon ICV injection or during a 7-day ICV infusion. Administration of pyrogen-free saline (PFS) injection did not induce significant change in daytime FI of control rats. Except for the oldest animals, the acute orexigenic effect of a central NPY injection on 1-h FI as compared with age-matched PFS-treated controls was significant and changed with aging (Fig. 2a). The amount of NPY-induced chow of the 12-month-old animals was significantly higher than those of 6-, 18-, 24-months-old ones. The oldest rats consumed less than the three youngest age groups (Fig. 2a). Since FI and BW did not change proportionally during aging (Fig. 1)[24,25], neither the absolute amount of FI nor FI relative to BW seems to be appropriate for direct comparison of the orexigenic effects of NPY among different age groups. Therefore, we applied the ratio of NPY-induced cumulative FI to the corresponding spontaneous daily FI (provided in Fig. 1e) and found significantly higher effect in 12-month-old than all other age groups (Fig. 2b). Compared to the young adult 3-month-old rats, the effect of the peptide showed an increase in middle-aged animals reaching a peak in the 12-month-old group, then it decreased in older animals. Thus, the age-related rise in the orexigenic responsiveness to NPY occurred before the peaks of BW and adiposity observed at 18 months, whereas the declined responsiveness of the 18-month-old group precedes the appearance of weight loss and sarcopenia by 24 months. The feeding activity was maximal in the first 60-min period following the NPY injection. This hyperphagia was followed by a rebound anorexia, which attenuated nocturnal feeding and resulted even in a slight transient weight loss (restricted to the first 24 h). Earlier, we have attributed this rebound anorexia to secondary anorexigenic mechanisms[26]. This change in 24-h BW expressed as % of initial BW was significant only in 3- and 12-month-old rats ($-3.4 \pm 0.9\%$ and $-2.5 \pm 0.9\%$, $p = 0.004$, 0.036, respectively, one-sample t-test).

Effects of chronic 7-day ICV infusion of NPY on daily FI, BW and the circadian changes in heart rate (HR), core temperature (Tc), spontaneous horizontal locomotor activity were measured in our biotelemetric system. Control animals treated with PFS did not change their FI, HR, Tc, activity during the tested 7-day period from day 1 (except for day 0 due to surgery) (Supplementary Figs. 1–4). The pre-infusion (baseline) values did not differ in NPY- vs. age-matched PFS-treated groups. Infusion of NPY significantly increased the daily FI in all age-groups as compared with their age-matched controls (Supplementary Fig. 1). Except for the 24-month-old group, we observed significant differences between NPY-treated rats and their age-matched controls during the whole infusion period. The hyperphagia lasted only for 4 days in the oldest animals (Supplementary Fig. 1).

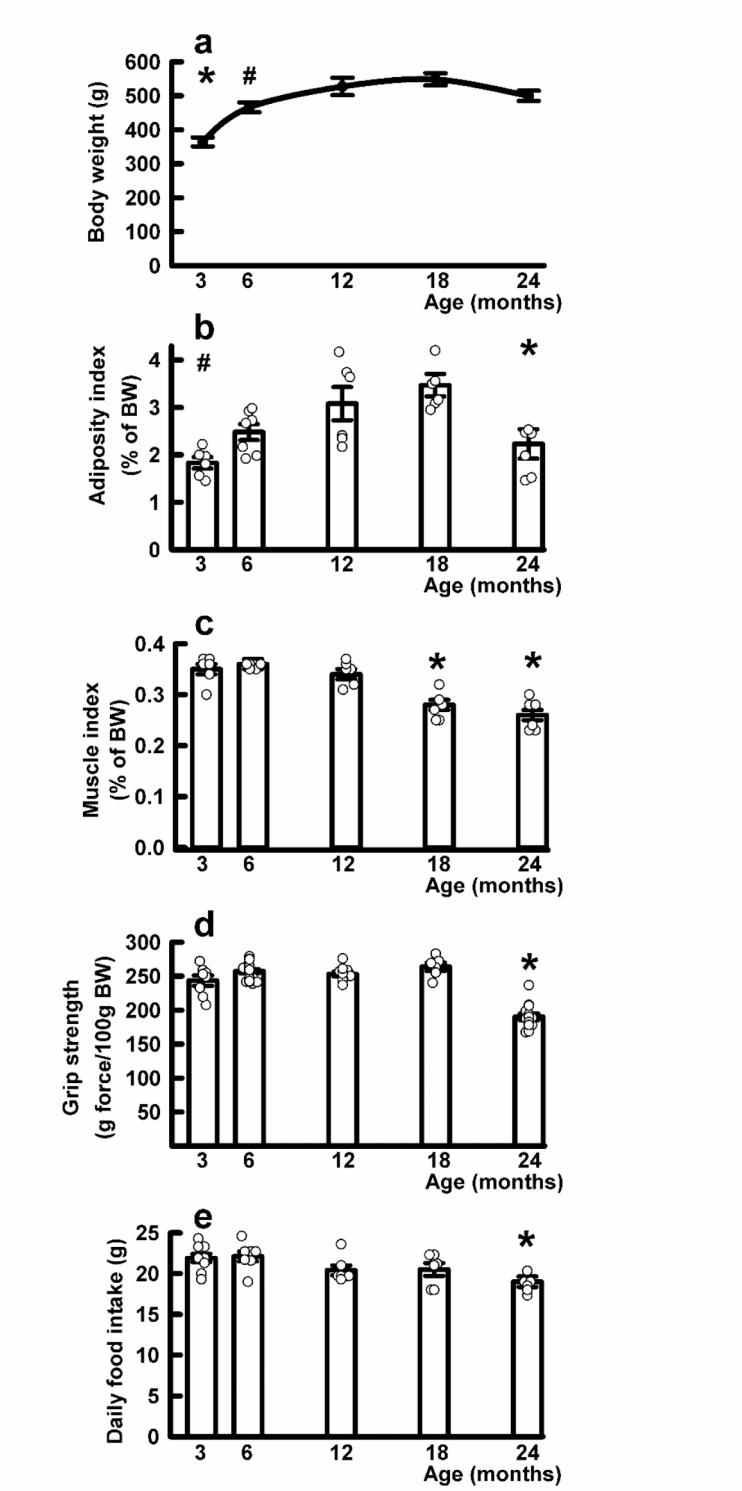


Fig. 1. Age-related changes in body weight (BW, **a**) of male Wistar rats, and their adiposity index (**b**), muscle index (**c**), muscle strength (**d**) and daily food intake (**e**). All data in bar plots are expressed as mean ± S.E.M. ($n = 6–9$/group) and were analyzed by one-way ANOVA with Tukey's post hoc test. (**a**) * indicates significant differences between the 3-month vs. all other age groups ($p < 0.001$ in all cases); # indicates significant difference between the 6-month-old vs. 18-month-old group ($p = 0.045$). (**b**) # indicates significant difference between the 3-month-old vs. 12- or 18-month-old groups ($p = 0.014$ or 0.001, respectively), * indicates significant difference between the 24-month-old vs. 18-month-old group ($p = 0.015$). (**c**) * indicate significant differences between the 18- or 24-month-old vs. 3-, 6-, 12-month-old groups ($p < 0.001$ in all cases). (**d**) * indicates significant difference between the 24-month-old vs. all other age groups ($p < 0.001$ in all cases). (**e**) * indicates significant difference between the 24-month-old vs. 3- or 6-month-old groups ($p = 0.010$ or 0.008, respectively).

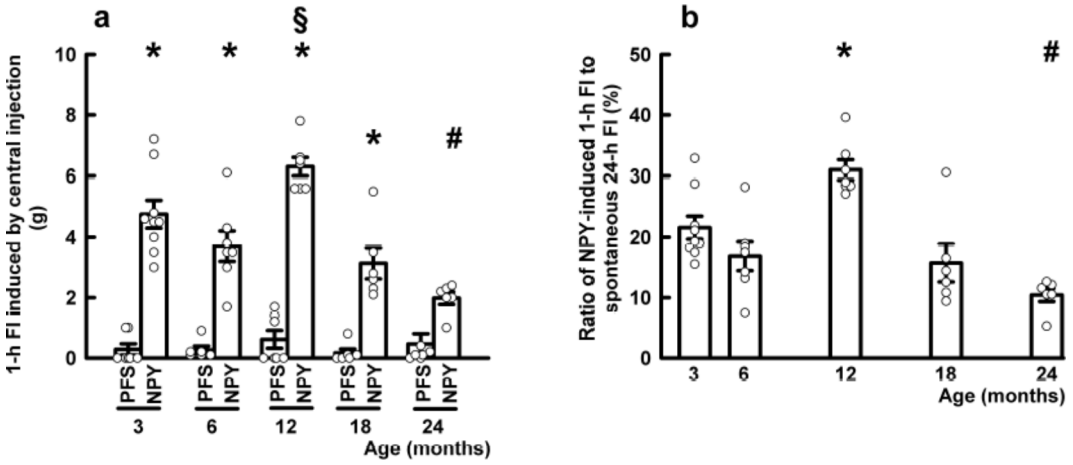


Fig. 2. Age-related changes in orexigenic responsiveness to central injection of NPY of male Wistar rats. Cumulative 1-h FI (food intake) induced by intracerebroventricularly injected NPY or pyrogen-free saline (PFS) expressed in g (**a**). NPY-induced FI of rats presented as ratio (%, **b**) to their own spontaneous 24-h FI (shown in Fig. 1e). All data in bar plots are expressed as mean ± S.E.M. ($n = 6–9$/group) and were analyzed by one-way ANOVA with Tukey's post hoc test. (**a**) * indicate significant differences between age-matched NPY- vs. PFS-treated groups ($p < 0.001$ in 3-, 6-, 12- and 18-month-old rats), § indicates significant differences between the 12 months vs. 6-, 18-, 24-month-old groups ($p < 0.001$ in all cases), # indicates significant difference between the 24-month-old vs. 3- or 6-month-old group ($p < 0.001$ or $p = 0.047$, respectively). (**b**) * indicates significant differences between the 12 months vs. 3-month-old or all other age groups ($p = 0.018$ or $p < 0.001$, respectively), # indicates significant difference between the 24-month-old vs. 3-month-old group ($p = 0.006$).

In order to analyze NPY-induced FI changes among different age groups, we compared mean daily FI during the NPY infusion (from day 1 to day 7) with the mean value of the pre-infusion daily FI (average for three pre-infusion days as own baseline) of the same animals in all age groups (Fig. 3a). Except for the oldest rats, this difference was significant in all age groups. The 12-month-old animals ate significantly more than the oldest ones ($p < 0.001$, one-way ANOVA with Tukey's post hoc test). The rate of increase in NPY-induced FI in relation to baseline (Fig. 3b) showed similar age-related pattern as seen in acute experiments (Fig. 2b) and it was significantly higher in the 12-month-old group than in other age groups. They raised their FI by 74% indicating a strong hyperphagic effect of NPY.

Chronic 7-day ICV infusion of NPY suppressed metabolic rate as shown by the reduced HR of the free-moving animals especially during the active nighttime period when their metabolic rate is already higher. The mean nighttime HR values (maxima of the circadian rhythm) decreased significantly in all age-groups as compared with their age-matched PFS-treated controls (Supplementary Fig. 2). This difference was significant during the first 4 days in the youngest group, and for 7 days in other age groups. This suppression of HR was strongest from day 1 to day 3. When comparing the mean of HR maxima on these first 3 days with the mean of their pre-infusion period (baseline HR) in each age group (Fig. 3c), the difference was significant only in 6-, 12- and 18-month-old groups. Baseline HR of the oldest rats was significantly lower than that of 6-month-old animals indicating a decline with aging ($p < 0.001$, one-way ANOVA with Tukey's post hoc test). The NPY-induced fall in relation to baseline (Fig. 3d) increased with age until 12 months (on some days reaching a maximal reduction exceeding 67 beats per minute) followed by a decline in the efficacy of NPY. Analysis of all groups by one-way ANOVA followed by Tukey's post hoc test did not show any significant age-related difference. However, when we compared only two groups directly by one-way ANOVA, HR reduction was significantly stronger in 12-month-old rats than in the 3- or the 24-month-old group ($p = 0.011$ or 0.049, respectively). Except for the 6-month-old rats, NPY infusion also induced a moderate, but significant reduction of the mean daytime HR (nadir of the circadian rhythm) in all age groups as compared with their age-matched PFS-treated controls (Supplementary Fig. 2). The difference was significant to day 5 in 3- and 24-month-old rats, the reduction remained significant throughout the 7-day period in 12- and 18-month-old groups. The suppression of the mean HR minima during the first 3 days (compared with their baseline values) seemed most pronounced in the oldest three groups, but the Tukey's post hoc test failed to show statistically significant age-related differences ($p > 0.05$). A direct comparison of the HR reduction in 12-month-old rats with the 3-month-old group revealed a significantly stronger suppression (-19 ± 6 vs. -3 ± 2 beats per minute, $p = 0.004$, one-way ANOVA). The hypometabolic effect of NPY assessed indirectly by the reduction in HR resulted in hypothermia. The mean nighttime Tc decreased significantly only in 3-, 12- and 18-month-old groups as compared with their age-matched controls (Supplementary Fig. 3). This suppression of Tc maxima lasted for 7 days and it was most pronounced from day 2 to day 6. Compared to the mean value of the pre-infusion period (baseline nighttime Tc), mean Tc maxima on the days 2–6 of the NPY infusion were reduced in the 3- and the 12-month-old groups, this fall exceeded eventually 1 °C, it was more moderate in 18-month-old rats (Fig. 3e). The decrease in Tc in relation to baseline (Fig. 3f) was significantly larger at 3 or 12 months than at 24 months of age. Centrally administered NPY induced significant reduction of

the daytime Tc values (nadir of the circadian rhythm) from day 3 to day 7 of the infusion only in 3-month-old animals as compared with their age-matched controls (Supplementary Fig. 3).

During the NPY infusion the nighttime spontaneous horizontal locomotor activity failed to show significant changes in any group. Surprisingly, despite daytime hypometabolism and hypothermia, the NPY-treated 3- and 12-month-old animals showed significantly higher daytime activity than their age-matched controls (Supplementary Fig. 4). The difference remained significant throughout the 7-day period. Moreover, this change was associated with disrupted circadian rhythm in 12-month-old animals since their mean daytime activity values exceeded the nighttime ones. This phenomenon could result from their high feeding activity even during the daytime period.

In contrast to other parameters, changes in BW showed age-related differences even in PFS-treated controls (Supplementary Fig. 5). The youngest rats gained weight (from 389.3 ± 5.4 g to 397.5 ± 4.3 g by the 7th day) corresponding to their normal growth rate that was missing in all older animals. The surgery induced a marked weight loss in the three oldest age groups, their BW started to be normalized only after day 4. Baseline BW was similar in control and in age-matched animals treated with NPY (Supplementary Table). The overall anabolic (i.e. hyperphagic and hypometabolic) effects of NPY resulted in weight gain. Except for the 6-month-old rats, the NPY infusion significantly increased the BW in all age-groups as compared with their age-matched controls (Supplementary Fig. 5). This effect was most pronounced from day 4 to day 6. Comparison of the NPY-induced mean BW changes for days 4–6 in relation to baseline with those of PFS-infusion showed age-dependent effects (Fig. 4). In this case, the NPY-induced weight gain was significant only in 12-month-old animals. Thus, the anabolic effect of NPY shows biphasic pattern: it increases in middle-aged animals and promotes weight gain before the peak of adiposity observed at 18 months of age. Then the responsiveness of the 18-month-old group declines followed by weight loss at 24 months of age.

Aging-related dynamics in the Npy mRNA expression and NPY peptide content of the ARC

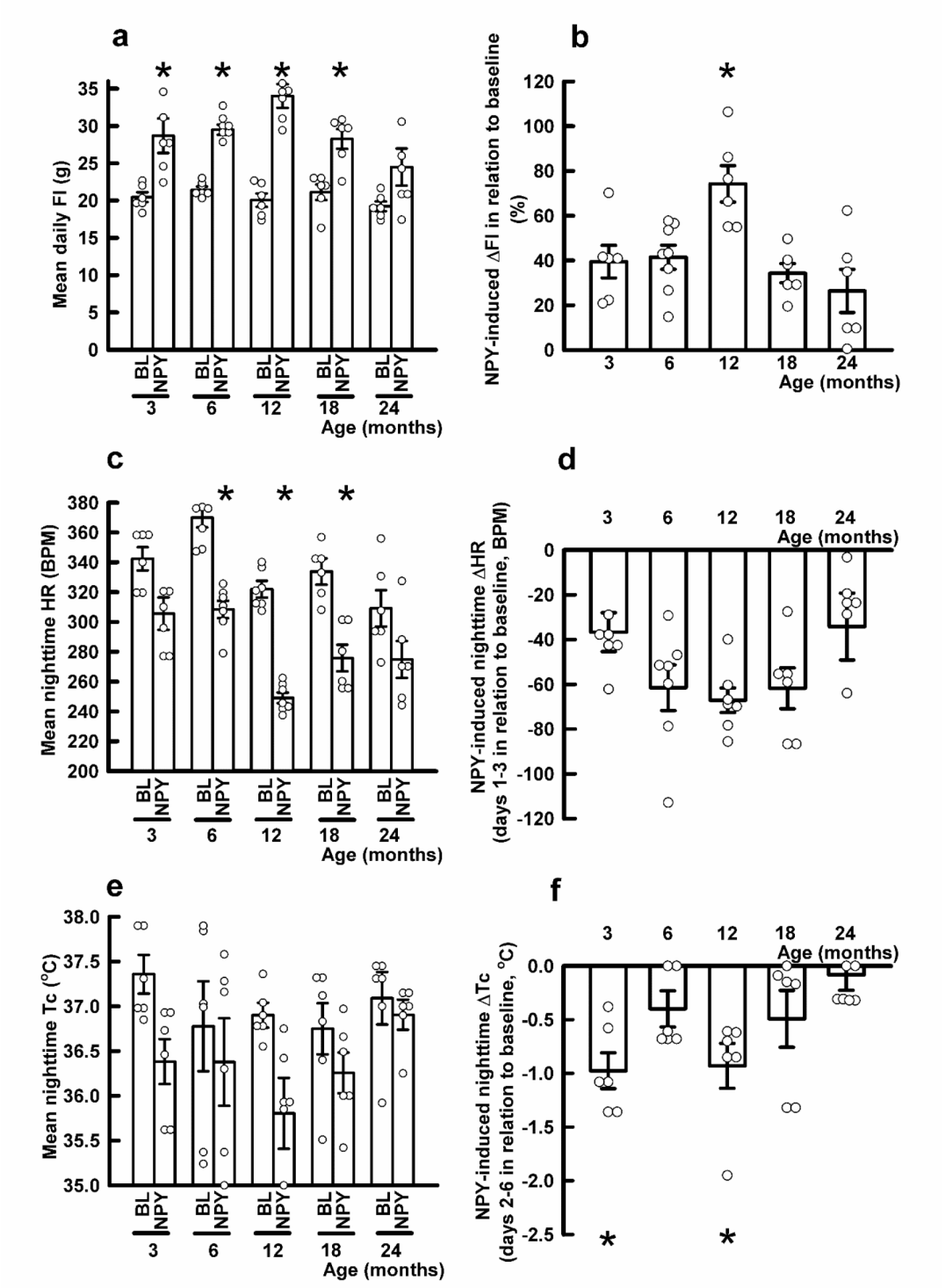
In accordance with our functional results, the RNAscope ISH combined with immunofluorescence revealed a significantly higher NPY specific signal strength density (SSD) value in the 12-month-old group than in younger 6-month-old and older 18- or 24-month-old rats (Fig. 5b). Thus, the highest values were found in the middle-aged animals. Interestingly, an opposite age-related dynamics (Fig. 5a) was observed in the Npy mRNA expression. A significant decrease in the younger middle-aged 6-month-old group was followed by a gradual increase with the course of aging.

Discussion

With regard to the endogenous activity and anabolic efficacy of central NPY in middle-aged groups, our study demonstrated different phases of aging and obesity in 6- and 12-month-old rats. Compared to the young adult animals, 6-month-old rats showed a maintained orexigenic responsiveness to NPY injection or infusion but decreased hypometabolic effects (significant decrease of HR but non-significant suppression of thermogenesis assessed by Tc) of NPY infusion. As a result, the anabolic NPY action on BW failed to develop only in this age group. Thus, the previously rapid growth was followed by a moderately rising slope of the BW development curve of our strain from 6 months. In contrast, in the older middle-aged 12-month-old group we found the highest orexigenic, hypometabolic (indicated by the suppression of HR and Tc), and overall anabolic (BW gain) effects of NPY infusion. The hyperphagic effect was so strong that it disrupted the circadian rhythm due to the high feeding-associated locomotor activity even during the inactive daytime period. As a result of the enhanced anabolic effectiveness of NPY the weight gain, especially the fat accumulation speeds up from 12 months reaching the peak by 18 months.

Endogenous central NPY activity was analyzed in vitro. Since we did not apply colchicine treatment to enhance immunosignal in the cell bodies, we quantified the specific SSD of NPY nerve fibers. Compared with the young adult group, the 6-month-old rats showed similar immunosignals in the ARC and significantly reduced Npy mRNA expression. In contrast, SSD in 12-month-old animals was the highest despite the lack of significant increase in Npy mRNA expression. Hyperleptinemia due to growing adiposity in 6-month-old animals could explain the reduced gene expression. Although hyperleptinemia is known to induce leptin resistance, leptin efficacy is still maintained to some extent in these rats[13], thus leptin may suppress the NPY synthesis. Reduced secretion or turnover rate could also contribute to this suppression and to unchanged SSD in nerve fibers. On the contrary, in more obese 12-month-old rats responsiveness to exogenous leptin is already strongly impaired[13,15]. Probably due to this leptin resistance the NPY gene expression is not decreased further. Indeed, the endogenous NPY system may exhibit enhanced activity and/or hypersensitivity as suggested by the high anabolic responsiveness to NPY in our in vivo experiments in ad libitum fed rats. Similarly, diet-induced obesity in young male Wistar rats also caused leptin resistance with a hypersensitivity to ICV NPY administration[27]. The animal models of obesity with a deficiency in leptin signaling (Zucker rat, Koletsky rat, db/db mouse) are characterized by an upregulation of the NPY system which contributes to their hyperphagia and excessive weight gain[28]. Our previous study on 12-month-old rats reported a suppressed activity of the melanocortin system[10] which is also known to inhibit NPY-positive neurons in the ARC and NPY-induced hyperphagia[29,30]. Thus, lacking inhibition of NPY by leptin and melanocortins may enhance NPY activity. One could speculate that NPY release may be slower, which leads to an accumulation of immunoreactivity. Alternatively, decreased elimination could also explain the high peptide content.

Changes in NPY receptors may also explain altered responsiveness to exogenous NPY. Reduced hypometabolic action of NPY in 6-month-old rats may be partly due to inhibited endogenous NPY release via Y2 autoreceptors of ARC NPY neurons[18] and/or to downregulation of Y1-Y5 receptors on second order neurons. The high responsiveness to NPY in 12-month-old rats may originate from NPY receptor up-regulation or hypersensitivity, but such changes have not been investigated in middle-aged obese animals.



Only two studies investigated the hyperphagic effect of central NPY injections in age groups also including middle-aged rats[31,32]. In contrast to our results, NPY-induced 4-h feeding following the injection to the third ventricle did not differ in 3-month-old and middle-aged 13-month-old Brown Norway (BN) rats[31]. However, 13-month-old BN males do not show such obesity as seen in our Wistar model. The BN strain is characterized by a gradual weight gain and fat accumulation until the age of 26–29 months reaching a modest 20–25% body fat. In contrast to aging men, this increase in fat mass occurs predominantly in peripheral and not visceral depots[33]. In contrast, our male Wistar rats show marked age-related weight gain and adiposity in the middle-aged group (12 months) reaching a peak at 18 months followed by a decline at 24 months. Another feature common with humans is their dominant visceral fat accumulation as we demonstrated earlier by microcomputer tomography[34]. The 13-month-old BN animals resemble rather our 6-month-old early middle-aged Wistar rats with moderate obesity and maintained orexigenic response to NPY similarly to young animals[31]. In the ARC of

◂**Fig. 3**. Effects of a 7-day intracerebroventricular NPY infusion on daily food intake (FI, **a** and **b**), nighttime heart rate (HR in beats per minute, BPM, **c** and **d**) and nighttime core temperature (Tc, **e** and **f**) in different age groups (aged 3, 6, 12, 18 or 24 months) of male Wistar rats. All data in bar plots are expressed as mean ± S.E.M. ($n = 6–8$/group) and were analyzed by one-way ANOVA with Tukey's post hoc test. (**a**) Mean daily FI before (baseline, BL) and during the NPY infusion (from day 1 to day 7). * indicate significant differences between baseline value and NPY-induced raise in 3-, 6-, 12- and 18-month-old age groups ($p = 0.006$, $p = 0.048$, $p < 0.001$, $p = 0.029$). (**b**) Rate of increase in mean daily FI induced by NPY infusion expressed in % of their pre-infusion baseline daily FI. * indicates significant differences between the 12 months vs. 3-, 6-, 18- and 24-month-old age groups ($p = 0.017$, 0.016, 0.005 and $p < 0.001$, respectively). (**c**) Mean of nighttime HR (12-h averaging for the active period) before (baseline, BL) and during the NPY infusion (on days 1–3). * indicate significant differences between baseline value and NPY-induced raise in 6-, 12- and 18-month-old age groups ($p < 0.001$ in all cases). (**d**) Rate of NPY-induced suppression in mean of nighttime HR compared with their pre-infusion baseline values. (**e**) Mean of nighttime Tc (12-h averaging for the active period) before (baseline, BL) and during the NPY infusion (on days 2–6). (**f**) Rate of NPY-induced suppression in mean of nighttime Tc compared with their pre-infusion baseline values. * indicate significant differences between the 3- or 12-month-old vs. 24-month-old groups ($p = 0.026$, 0.037, respectively).

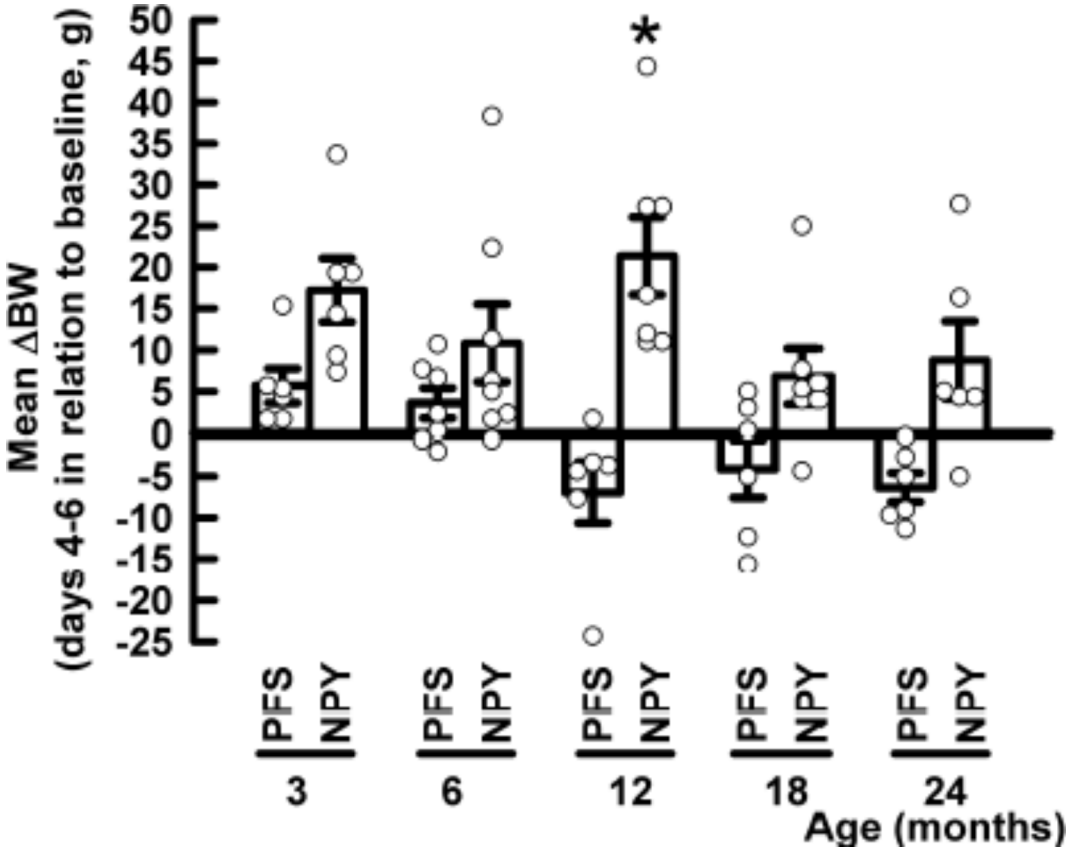


Fig. 4. Effects of a 7-day intracerebroventricular NPY infusion on body weight (BW) in different age groups (aged 3, 6, 12, 18 or 24 months) of male Wistar rats. All data in bar plots are expressed as mean ± S.E.M. ($n = 6–8$/group) and were analyzed by one-way ANOVA with Tukey's post hoc test. Changes in mean of BW on days 4–6 of the NPY infusion or pyrogen-free saline (PFS) are presented in relation to their baseline BW on day 0. * indicates significant difference between the 12-month-old NPY- vs. PFS-treated group ($p < 0.001$). Initial BW values in NPY-treated vs. their age-matched PFS-treated controls did not differ.

12-month-old BN rats significantly lower prepro-Npy mRNA was found than in young 3-month-old ones[35], that is similar to the reduction of Npy mRNA expression in our 6-month-old Wistar rats. Other researchers reported that 11-month-old male Wistar rats failed to increase FI significantly upon ICV injections of the same dose of NPY which was effective in young 4-month-old animals[32]. Nevertheless, the BW of this middle-aged group was markedly lower than that of our rats (335–465 g vs. 527 ± 25 g), thus they did not show the characteristic middle-aged obesity demonstrated in our strain.

In sum, progressive obesity in middle-aged animals is associated with high NPY peptide content of ARC and enhanced hyperphagic and hypometabolic effects of NPY further increasing adiposity (Figs. 2, 3, 4 and 5).

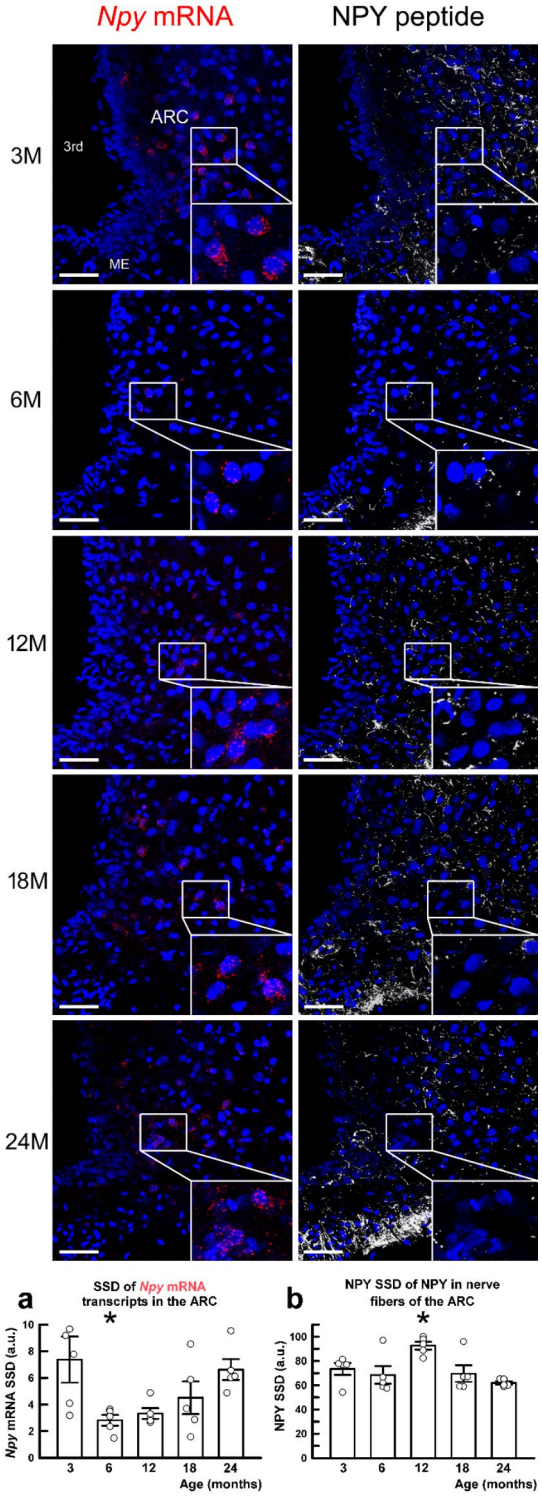


Fig. 5. Comparison of Npy mRNA expression and NPY peptide content of the arcuate nucleus (ARC) in 3-, 6-, 12-, 18- and 24-month-old (M) male rats. Representative confocal images show the Npy mRNA expression (red in the left images) by RNAscope in situ hybridization. All data in bar plots are expressed as mean ± S.E.M. (n = 5/group) and were analyzed by one-way ANOVA with Tukey's post hoc test. Panel (**a**) illustrates the results of the semiquantitative measurement of Npy mRNA specific signal density (SSD) expressed in arbitrary units (a.u.). * indicates significant difference between the 6-month-old vs. 3-month-old group (p = 0.04). On the right, images show the ARC/NPY SSD in nerve fibers, as visualized by immunofluorescence (white). Panel (**b**) illustrates the SSD of NPY peptide immunosignal in the course of aging. Blue: 4',6-diamidino-2-phenylindole (DAPI) nuclear counterstaining. * indicates significant differences between the 12-month-old vs. 6-, 18- or 24-month-old groups ($p = 0.028$, 0.039 or 0.004, respectively). 3rd: third ventricle. ME: median eminence. Bars: 50 µm.

Additionally, NPY decreases sympathetic activity shown by reduction of HR in our experiments. A decrease in oxygen consumption and thermogenesis is also suggested although hypothermic effects of central NPY could only be demonstrated earlier in cool environments where the metabolic rate is already elevated[26]. Our present study also showed NPY-induced suppression of thermogenesis during the active nighttime period characterized by hypermetabolism. NPY-induced sympathetic inhibition also suppresses lipolysis and increases adipogenesis in white adipose tissue. These changes result in peak adiposity and BW development in aging 18-month-old Wistar rats. The importance of our findings is further underlined by the observation that NPY-knockout male mice failed to develop aging-induced weight gain and adiposity[36].

We aimed to clarify age-related dynamics in NPY activity at multiple stages of life. We compared five different age groups from young adult to old. We are the first to reveal that the NPY system shows non-linear changes with aging: first a peak developed in middle-aged 12-month-old rats followed by a gradual decline in the two oldest groups. Compared with young rats, we confirmed the blunted hyperphagic effect of ICV NPY injections in very old rats with terminal weight loss also described by earlier studies[31,32,37]. However, these reduced NPY effects were preceded by an enhancement in the middle-aged group and a decline in 18-month-old rats before the onset of aging anorexia in our oldest group. In 18-month-old animals with the highest body fat both hyperphagic and hypometabolic effects started to decline, therefore the action on BW also decreased. SSD of NPY nerve fibers in the ARC of this group was also reduced compared with the 12-month-old group. The Npy mRNA expression changed in the opposite direction (Fig. 5a), but this increase was rather a tendency and did not reach statistical significance. Earlier studies compared the hypothalamic NPY content and gene expression only in old and young rats and they found significantly lower levels in the old group[8,35,38,39]. We could explain the decline in NPY activity by an enhancement of inhibitory mechanisms by leptin and/or melanocortins in aging and old animals. Indeed, at 18 months of age we described a significant rise in the expression of the long form of the leptin receptor in the ARC compared to the middle-aged group[15] and an increased activity of the melanocortin system, as well[10]. The contribution of NPY receptors was investigated in 27-month-old Fischer 344 rats[40]. The altered responsiveness of very old rats to NPY could not be explained by decreased production of Y1–Y5 receptors in the paraventricular nucleus. Changes in the signal transduction of NPY receptors may be responsible for aging-induced altered sensitivity to NPY.

We performed a detailed analysis of age-related changes in muscle mass and strength. Earlier data based on the measurement of tibialis anterior muscle in male Wistar rats[11] or sarcopenia index defined by soleus muscle weight in Sprague-Dawley rats[41] showed stable muscle mass from young adult to middle-aged groups followed by a decline in very old age. We calculated a new muscle index relative to BW by measurement of wet weights of tibialis anterior, soleus muscles and the extensor digitorum longus and extensor hallucis longus muscles. In line with earlier observations, our muscle index remained unchanged from young adult to late middle-aged groups (12-month-old) demonstrating proportional increases in muscle mass and BW (Fig. 1a, c) and showed significant muscle loss with decreased muscle strength in very old animals indicating aging sarcopenia based on reduced daily food intake reflecting aging anorexia[11,41,42]. As a new finding, our muscle index started to decline earlier than muscle strength already at 18 months, similarly to the observations in corresponding human age groups[43]. Accordingly, from 12 months, only body fat increases further resulting in adiposity combined with age-related muscle loss in 18-month-old animals (Fig. 1). These findings suggest that loss of muscle mass leading to aging sarcopenia appears before the onset of age-related reduction in muscle strength and aging anorexia (seen in our very old rats).

Inflammatory mediators released from excess adipose tissue[5] in the 18-month-old group may contribute to the age-related damage of muscles. This muscle loss is further aggravated by aging anorexia in the oldest 24-month-old animals due to lack of orexigenic NPY and dominance of anorexigenic mediators (leptin, melanocortins). Moreover, decreased suppression of sympathetic activity by NPY increases lipolysis and decreases adipogenesis. These may promote ectopic fat accumulation in muscles leading to further impairment and sarcopenia. At old age, they may result in some decline in body fat.

Human aging is associated with alterations in the regulation of energy balance and body weight, characterized by reduced spontaneous FI (anorexia of aging)[44] and impaired FI and BW recovery after fasting (dysorexia)[45]. Reduced NPY orexigenic responsiveness may contribute to these changes. Moreover, our novel findings suggest reduced metabolic responsiveness to NPY in old age. This apparent insensitivity to metabolic cues can lead to inappropriate weight loss in response to acute or chronic illness or other stressors, resulting in greater morbidity and mortality in geriatric populations[46]. Therefore, stimulation of the central NPY system (e.g. by potent central NPY receptor agonists) may have beneficial effects in aging anorexia since NPY[21] promotes FI with a preferential carbohydrate intake and increases carbohydrate utilization as energy source leading to an increase in respiratory quotient[22,28,47,48]. In addition, pharmacological restoration of the metabolic activity of NPY in older individuals may decrease lipolysis, the release of fatty acids and thereby ectopic fat accumulation in aging muscles. Thus, stimulation of the NPY system could be a promising therapeutic target in line with recent studies on anti-aging effects of NPY[21]. However, beneficial effects of NPY in patients with aging obesity could be questioned. Indeed, selective NPY antagonists present great potential as pharmacological targets for the treatment of obesity[49,50]. Our novel observation revealed a hyperactive NPY system in middle-aged animals with increasing adiposity suggesting a role of NPY in age-related obesity. Our results highlight the importance of age-related differences in anabolic responsiveness to NPY especially with regard to the doses of future drugs.

Methods

Animals

Male Wistar rats from the colony of the Institute for Translational Medicine, University of Pécs, Hungary were used: 3, 6, 12, 18, and 24 months of age, corresponding to human young adult, younger or older middle-aged, aging, and old populations, respectively. The maximal life-span of our colony reaches 30 months, about 50% of

rats survive 26 months, but after the age of 24 months surgical interventions are difficult[15]. They were housed individually in plastic cages (42.7 cm × 26.7 cm × 18 cm) with woodchip bedding covered with steel grids. Standard rat chow (11 kJ/g; CRLT/N, Szindbád Kft., Hungary) and tap water were available ad libitum. Animals were kept under a 12 h/12 h dark/light cycle and under an ambient temperature range of 23–25 °C. All animals were accustomed to regular handling, daily measurement of their BW and to the experimental conditions. Two cohorts were subjected to ICV cannula implantation for in vivo investigation of the effects of centrally injected (acute experiments) or infused (chronic experiments) NPY or pyrogen-free saline (PFS) as control. All groups contained 6–9 rats. Another cohort of intact rats ($n = 6–7$/age group) was used for measurement of muscle strength. One week later, the brains of these animals were collected for RNAscope in situ hybridization (ISH) combined with immunohistochemistry. Their body composition was analyzed post mortem.

Our protocols and procedures were approved by the Animal Welfare Committee of the University of Pécs and by the National Scientific Ethical Committee on Animal Experimentation of Hungary. The license was granted by the Government Office of Baranya County (BA02/2000-6/2020). They were also in accordance with the directives of the European Union (86/609/EEC, Directive2010/63/EU) and the rules of the Hungarian Government (40/2013.II.14.) on the protection of animals used for scientific purposes. This study was reported in accordance with the ARRIVE guidelines.

Surgeries and drug administration

Upon reaching the appropriate age, 22-gauge stainless-steel guide cannula were implanted into the right lateral cerebral ventricle under intraperitoneal ketamine + xylazine [78 mg/kg (Calypsol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia. Rats were treated by intramuscular gentamycin (2 mg/kg) to prevent postoperative infections. The implantation was performed using a stereotaxic apparatus as described earlier[11].

Acute experiments started 7 days after the cannula implantation. The placement of the cannula was checked by the ICV injection of angiotensin II 2 days before the tests as described earlier[11]. During the tests, a single 5 µl ICV injection of 5 µg NPY (Bachem AG Switzerland, NPY 1–36, MW 4271.74, Cat No: H-6375) dissolved in PFS or PFS as control was given in random order as described earlier[11]. After 7 days, the substances were switched and the measurements were repeated. All applied doses were chosen based on our earlier observations[26,51].

For the chronic tests, at least one week before the ICV cannula implantation the rats underwent the implantation of biotelemetric transmitters (MiniMitter VMFH, series 4000, Sunriver) into the peritoneal cavity under intraperitoneal anesthesia as previously described[12]. The ICV cannula (Alzet Brain Infusion Kit) was connected to an Alzet osmotic minipump (model 2001) which was placed under the skin of the neck and contained solution for a 7-day-long infusion (1 µg/µl/h NPY or 1 µl/h PFS in control animals). This operation was performed on day 0 between 09.00 and 15.00 h. This severely influenced all parameters even in PFS-treated animals[12]. Except for BW, other parameters were practically normalized in all age groups during the night following the surgery. Comparisons between control and NPY-infused groups were possible from day 1. Only BW decreased on day 1 due to the low FI on the previous day.

After the experiments, rats were euthanized by an intraperitoneal overdose of urethane (3–5 g/kg, Reanal). Post mortem check of the injection sites was performed by observing macroscopically the coronal sections of the removed brains. Only rats with appropriate cannula location were included in the analysis.

Assessment of acute orexigenic effects of NPY

Two weeks before the experiments, rats were transferred individually to chambers of the automated FeedScale system (Columbus, OH, USA). Powdered chow was used in order to avoid hoarding. Food consumption was recorded every 10 min. After the ICV NPY or PFS injections (at 09.00 h), the follow-up lasted for 24 h.

Assessment of chronic effects of NPY on energy homeostasis

In our biotelemetric system, the implanted transmitters recorded Tc, HR (for indirect assessment of metabolic rate) and spontaneous horizontal locomotor activity continuously in freely moving animals. The receiver was placed under the MiniMitter cage. Data were sampled every 5 min and averaged for 12 h periods by the computer (VitalView software). One mean value was generated for the night (active period) and one for the daytime (inactive period). Spontaneous circadian changes were observed for about 1 week after transmitter implantation then the ICV infusion cannula with osmotic minipump were implanted. After waking up from the anesthesia, the rats resumed their activities in their cages. Daily FI and BW were measured manually.

Assessment of age-related differences in muscle strengths of intact rats

For the assessment of skeletal muscle strength a grip strength meter for rats (model 47200; Ugo Basile, Italy) was used. A rat, held by the tail, was allowed to reflexively grasp with both forelimbs the T-shaped bar attached to the force transducer. The experimenter pulled the rat by the tail gently till the animal lost the grip. The maximum force generated before the loss of grip was automatically registered. The data were analyzed using DCA software (version1.1, Ugo Basile, Italy). Forelimb grip strength scores were obtained by averaging the force (g) of three readings for each animal, taken by the same two researchers to decrease variation. The score was adjusted to body weight of the rat (g/100 g BW).

Post mortem body composition analysis

Body composition of intact rats euthanized for in vitro tests were determined to reveal age-related differences. Adiposity index was assessed by the measurement of bilateral epididymal and retroperitoneal fat pads and expressed as % of actual BW[52]. To obtain an indicator of muscle mass we introduced a novel muscle index: the left tibialis anterior, soleus, extensor digitorum longus, and extensor hallucis longus muscles were removed, and

their wet weights were calculated for 100 g BW. Previous studies have employed some of these muscles for the study of age-related sarcopenia[11,42].

RNAscope ISH combined with immunofluorescence

An independent cohort of naive male Wistar rats ($n = 5$/age group with BW similar to age-matched animals of the in vivo experiments) was intraperitoneally anesthetized (urethane, 2.8 g/kg, Merck KGaA, Darmstadt, Germany), transcardially perfused with 50 mL 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by 250 mL 4% paraformaldehyde in Millonig's buffer. Brains were dissected, post-fixed and five series of thirty µm coronal Vibratome (Leica Biosystems, Wetzlar, Germany) sections between − 1.5 mm and − 3.5 mm to the bregma[53] were collected and stored in anti-freeze solution at − 20 °C. Four representative ARC sections per animal were selected and subjected to a modified pretreatment for RNAscope ISH, optimized for 30 µm sections, as we recently published[54]. Subsequent steps of the RNAscope were performed according to the supplier's suggestions. Npy mRNA was visualized by Cy3 (1:3000) using a rat Npy probe (Cat No: 450971-C2, Advanced Cell Diagnostics, Newark, CA, USA, ACD). After channel development, the sections were rinsed with PBS and treated with a polyclonal sheep NPY antiserum (1:48:000, FJL #14/3A, generous gift of Dr. Istvan Merchenthaler) overnight, at room temperature. After PBS washes, an Alexa Fluor 647-conjugated donkey anti-sheep secondary antiserum (1:500, RRID: AB_2340750, Cat No: 713-605-003, Jackson Immunoresearch Europe Ltd., Cambridgeshire, UK) was applied for 3 h. Finally, after washes, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and covered with antifade medium. The sensitivity of the test was confirmed in randomly selected ARC sections, hybridized with triplex positive (Cat No: 320891, ACD) and negative control (Cat No: 320871, ACD) probes. The positive control emitted obvious cytoplasmic fluorescence, while no signal puncta were seen in the negative control. The specificity and sensitivity of the NPY serum in the rat brain tissue was tested earlier by others[55] and in our laboratory[56]. No immunosignal was seen in sections where the primary serum was omitted or replaced with normal sheep serum.

Four ARC cross-section areas per animal were digitalized using Olympus FluoView 1000 confocal microscope with 40x (NA:0.8) objective. The excitation and emission of fluorophores were chosen according to the built-in settings of the FluoView software (Fv10-ASW; Version0102). Blue (DAPI), red (Cyanine 3) and white (Alexa Fluor 647) virtual colors were assigned to the dyes. 1024 × 1024 pixel images were taken in sequential scanning mode with an optical thickness of 3.5 µm. The density of cytoplasmic Npy mRNA signal dots was measured in five ideally cut ARC cells, per section. The density of the NPY immunoreactivity was also measured, but because the cell bodies do not contain reliably detectable amount of NPY peptide without slowing down the axonal transport by colchicine treatment[56], the assessment was performed in the NPY-containing nerve fibers in the ARC. Four non-edited digital images per animal by two independent researchers (D.K.K. and Sz.E.) were quantified using the ImageJ software (version 1.52a, NIH). The four values were averaged, and this number represented one animal in the statistics. For publication, selected representative images were cropped, contrasted using Adobe Photoshop software.

Statistical analysis

Repeated-measures ANOVA or one-way ANOVA with Tukey's post hoc tests were used (SPSS for Windows 25.0). One-sample t-test was applied for the analysis of 24-h BW lost upon NPY injection. The significance was set at the level of $p < 0.05$. SigmaPlot 11.0 software was used to create the graphics. All results are shown as mean ± standard error of mean (S.E.M.).

Data availability

The data used to support the findings of this paper are available from the corresponding author upon reasonable request.

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References

1. Ding, J. et al. Effects of birth cohort and age on body composition in a sample of community-based elderly. *Am. J. Clin. Nutr.* **85** (2), 405–410. https://doi.org/10.1093/ajcn/85.2.405 (2007).
2. Kelly, T. L., Wilson, K. E. & Heymsfield, S. B. Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS One*. **4** (9), e7038. https://doi.org/10.1371/journal.pone.0007038 (2009).
3. Roubenoff, R., Dallal, G. E. & Wilson, P. W. Predicting body fatness: the body mass index vs estimation by bioelectrical impedance. *Am. J. Public. Health*. **85** (5), 726–728. https://doi.org/10.2105/ajph.85.5.726 (1995).
4. Palmer, B. F. & Clegg, D. J. The sexual dimorphism of obesity. *Mol. Cell. Endocrinol.* **402**, 113–119. https://doi.org/10.1016/j.mce.2014.11.029 (2015).
5. Stenholm, S. et al. Sarcopenic obesity: definition, cause and consequences. *Curr. Opin. Clin. Nutr. Metab. Care*. **11** (6), 693–700. https://doi.org/10.1097/MCO.0b013e328312c37d (2008).
6. JafariNasabian, P., Inglis, J. E., Reilly, W., Kelly, O. J. & Ilich, J. Z. Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake. *J. Endocrinol.* **234** (1), R37–R51. https://doi.org/10.1530/JOE-16-0603 (2017).
7. Coelho-Júnior, H. J., Marques, F. L., Sousa, C. V., Marzetti, E. & Aguiar, S. D. S. Age- and sex-specific normative values for muscle mass parameters in 18,625 Brazilian adults. *Front. Public. Health*. **11**, 1287994. https://doi.org/10.3389/fpubh.2023.1287994 (2024).
8. Scarpace, P. J. & Tümer, N. Peripheral and hypothalamic leptin resistance with age-related obesity. *Physiol. Behav.* **74** (4–5), 721–727. https://doi.org/10.1016/s0031-9384(01)00616-3 (2001).
9. Székely, M., Soós, S., Pétervári, E. & Balaskó, M. Academic Press, Nutritional impact on anabolic and catabolic signaling. Chapter 14 in book *Molecular Basis of Nutrition and Aging* (eds. Malavolta, M., Mocchegiani, E) 189–204 (2016). https://doi.org/10.1016/B978-0-12-801816-3.
10. Füredi, N. et al. Activity of the hypothalamic melanocortin system decreases in middle-aged and increases in old rats. *J. Gerontol. Biol. Sci. Med. Sci.* **73** (4), 438–445. https://doi.org/10.1093/gerona/glx213 (2018).

11. Pétervári, E., Garami, A., Soós, S., Székely, M. & Balaskó, M. Age-dependence of alpha-MSH-induced anorexia. *Neuropeptides*. **44** (4), 315–322. https://doi.org/10.1016/j.npep.2010.03.002 (2010).
12. Pétervári, E. et al. Central alpha-MSH infusion in rats: disparate anorexic vs. metabolic changes with aging. *Regul. Pept.* **166** (1–3), 105–111. https://doi.org/10.1016/j.regpep.2010.10.002 (2011).
13. Pétervári, E. et al. Age versus nutritional state in the development of central leptin resistance. *Peptides*. **56**, 59–67. https://doi.org/10.1016/j.peptides.2014.03.011 (2014).
14. Rostás, I. et al. Age-related alterations in the central thermoregulatory responsiveness to alpha-MSH. *J. Therm. Biol.* **49–50**, 9–15. https://doi.org/10.1016/j.jtherbio.2015.01.004 (2015).
15. Rostás, I. et al. Age-related changes in acute central leptin effects on energy balance are promoted by obesity. *Exp. Gerontol.* **85**, 118–127. https://doi.org/10.1016/j.exger.2016.10.006 (2016).
16. Tenk, J. et al. Acute central effects of corticotropin-releasing factor (CRF) on energy balance: effects of age and gender. *Peptides*. **85**, 63–72. https://doi.org/10.1016/j.peptides.2016.09.005 (2016).
17. Koebele, S. V. & Bimonte-Nelson, H. A. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas*. **85**, 5–17. https://doi.org/10.1016/j.maturitas.2016.01.015 (2016).
18. Zhang, W., Cline, M. A. & Gilbert, E. R. Hypothalamus-adipose tissue crosstalk: neuropeptide Y and the regulation of energy metabolism. *Nutr. Metab. (Lond)*. **11** (27). https://doi.org/10.1186/1743-7075-11-27 (2014).
19. Nguyen, A. D. et al. Y1 and Y5 receptors are both required for the regulation of food intake and energy homeostasis in mice. *PLoS One*. **7** (6). https://doi.org/10.1371/journal.pone.0040191 (2012). e40191.
20. Shi, Z., Madden, C. J. & Brooks, V. L. Arcuate neuropeptide Y inhibits sympathetic nerve activity via multiple neuropathways. *J. Clin. Investig.* **127** (7), 2868–2880. https://doi.org/10.1172/JCI92008 (2017).
21. Botelho, M., Cavadas, C. & Neuropeptide, Y. An anti-aging player? *Trends Neurosci.* **38** (11), 701–711. https://doi.org/10.1016/j.tins.2015.08.012 (2015).
22. Su, Y., Foppen, E., Fliers, E. & Kalsbeek, A. Effects of intracerebroventricular administration of neuropeptide Y on metabolic gene expression and energy metabolism in male rats. *Endocrinology*. **157** (8), 3070–3085 (2016). 10.1210/en.2016 – 1083.
23. Baran, K. et al. Chronic central melanocortin-4 receptor antagonism and central neuropeptide-Y infusion in rats produce increased adiposity by divergent pathways. *Diabetes*. **51** (1), 152–158. https://doi.org/10.2337/diabetes.51.1.152 (2002).
24. Kmieć, Z., Pétervári, E., Balaskó, M. & Székely, M. Anorexia of aging. *Vitam. Horm.* **92**, 319–355. https://doi.org/10.1016/B978-0-12-410473-0.00013-1 (2013).
25. Toshinai, K. et al. Ghrelin stimulates growth hormone secretion and food intake in aged rats. *Mech. Ageing Dev.* **128** (2), 182–186. https://doi.org/10.1016/j.mad.2006.10.001 (2007).
26. Székely, M., Pétervári, E., Pákai, E., Hummel, Z. & Szelényi, Z. Acute, subacute and chronic effects of central neuropeptide Y on energy balance in rats. *Neuropeptides*. **39** (2), 103–115. https://doi.org/10.1016/j.npep.2005.01.005 (2005).
27. van den Heuvel, J. K. et al. Neuropeptide Y and leptin sensitivity is dependent on diet composition. *J. Neuroendocrinol.* **26** (6), 377–385. https://doi.org/10.1111/jne.12155 (2014).
28. Beck, B. Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philos. Trans. R Soc. Lond. B: Biol. Sci.* **361** (1471), 1159–1185. https://doi.org/10.1098/rstb.2006.1855 (2006).
29. Domingo, P., Villarroya, F., Giralt, M. & Domingo, J. C. Potential role of the melanocortin signaling system interference in the excess weight gain associated to some antiretroviral drugs in people living with HIV. *Int. J. Obes. (Lond)*. **44** (9), 1970–1973. https://doi.org/10.1038/s41366-020-0551-5 (2020).
30. Murphy, B. et al. Melanocortin mediated inhibition of feeding behavior in rats. *Neuropeptides*. **32** (6), 491–497. https://doi.org/10.1016/s0143-4179(98)90077-4 (1998).
31. Wolden-Hanson, T., Marck, B. T. & Matsumoto, A. M. Blunted hypothalamic neuropeptide gene expression in response to fasting, but preservation of feeding responses to AgRP in aging male Brown Norway rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287** (1), R138–R146. https://doi.org/10.1152/ajpregu.00465.2003 (2004).
32. Akimoto-Takano, S. et al. Differences in the appetite-stimulating effect of orexin, neuropeptide Y and ghrelin among young, adult and old rats. *Neuroendocrinology*. **82** (5–6), 256–263. https://doi.org/10.1159/000092754 (2005).
33. Wolden-Hanson, T., Marck, B. T., Smith, L. & Matsumoto, A. M. Cross-sectional and longitudinal analysis of age-associated changes in body composition of male Brown Norway rats: association of serum leptin levels with peripheral adiposity. *J. Gerontol. Biol. Sci. Med. Sci.* **54** (3), B99–B107. https://doi.org/10.1093/gerona/54.3.b99 (1999).
34. Tekus, E. et al. Body fat of rats of different age groups and nutritional states: assessment by micro-CT and skinfold thickness. *J. Appl. Physiol. (1985)*. **124** (2), 268–275. https://doi.org/10.1152/japplphysiol.00884.2016 (2018).
35. Gruenewald, D. A., Marck, B. T. & Matsumoto, A. M. Fasting-induced increases in food intake and neuropeptide Y gene expression are attenuated in aging male brown Norway rats. *Endocrinology*. **137** (10), 4460–4467. https://doi.org/10.1210/endo.137.10.8828508 (1996).
36. Park, S. et al. NPY antagonism reduces adiposity and attenuates age-related imbalance of adipose tissue metabolism. *FASEB J.* **28** (12), 5337–5348. https://doi.org/10.1096/fj.14-258384 (2014).
37. Blanton, C. A. et al. Reduced feeding response to neuropeptide Y in senescent Fischer 344 rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280** (4). https://doi.org/10.1152/ajpregu.2001.280.4.R1052 (2001). R1052-R1060.
38. Kowalski, C., Micheau, J., Corder, R., Gaillard, R. & Conte-Devolx, B. Age-related changes in cortico-releasing factor, somatostatin, neuropeptide Y, methionine enkephalin and beta-endorphin in specific rat brain areas. *Brain Res.* **582** (1), 38–46. https://doi.org/10.1016/0006-8993(92)90314-y (1992).
39. Pich, E. M. et al. Feeding and drinking responses to neuropeptide Y injections in the paraventricular hypothalamic nucleus of aged rats. *Brain Res.* **575** (2), 265–271. https://doi.org/10.1016/0006-8993(92)90089 (1992).
40. Coppola, J. D., Horwitz, B. A., Hamilton, J. & McDonald, R. B. Expression of NPY Y1 and Y5 receptors in the hypothalamic paraventricular nucleus of aged Fischer 344 rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287** (1), R69–R75. https://doi.org/10.1152/ajpregu.00607.2003 (2004).
41. Edström, E. & Ulfhake, B. Sarcopenia is not due to lack of regenerative drive in senescent skeletal muscle. *Aging Cell.* **4** (2), 65–77. https://doi.org/10.1111/j.1474-9728.2005.00145.x (2005).
42. Rice, K. M., Linderman, J. K., Kinnard, R. S. & Blough, E. R. The Fischer 344/NNiaHSd X Brown Norway/BiNia is a better model of Sarcopenia than the Fischer 344/NNiaHSd: a comparative analysis of muscle mass and contractile properties in aging male rat models. *Biogerontology*. **6** (5), 335–343. https://doi.org/10.1007/s10522-005-4808-0 (2005).
43. Cheng, S. J., Yang, Y. R., Cheng, F. Y., Chen, I. H. & Wang, R. Y. The changes of muscle strength and functional activities during aging in male and female populations. *Int. J. Gerontol.* **7** (4), 231–235. https://doi.org/10.1016/j.ijge.2013.08.014 (2014).
44. Rolls, B. J., Dimeo, K. A. & Shide, D. J. Age-related impairments in the regulation of food intake. *Am. J. Clin. Nutr.* **62** (5), 923–931. https://doi.org/10.1093/ajcn/62.5.923 (1995).
45. Roberts, S. B. et al. Control of food intake in older men [published correction appears in JAMA 1995;273(9):702]. *JAMA*. **272** (20), 1601–1606. https://doi.org/10.1001/jama.1994.03520200057036 (1994).
46. Merchant, R. A., Woo, J., Morley, J. E. & Editorial Anorexia of Ageing: pathway to Frailty and Sarcopenia. *J. Nutr. Health Aging*. **26** (1), 3–5. https://doi.org/10.1007/s12603-021-1703-6 (2022).
47. Currie, P. J. & Coscina, D. V. Regional hypothalamic differences in neuropeptide Y-induced feeding and energy substrate utilization. *Brain Res.* **737** (1–2), 238–242. https://doi.org/10.1016/0006-8993(96)00738-x (1996).

48. Menéndez, J. A., McGregor, I. S., Healey, P. A., Atrens, D. M. & Leibowitz, S. F. Metabolic effects of neuropeptide Y injections into the paraventricular nucleus of the hypothalamus. *Brain Res.* **516** (1), 8–14. https://doi.org/10.1016/0006-8993(90)90890-n (1990).
49. Yulyaningsih, E., Zhang, L., Herzog, H. & Sainsbury, A. NPY receptors as potential targets for anti-obesity drug development. *Br. J. Pharmacol.* **163** (6), 1170–1202. https://doi.org/10.1111/j.1476-5381.2011.01363.x (2011).
50. Yi, M. et al. A promising therapeutic target for metabolic diseases: Neuropeptide Y receptors in humans. *Cell. Physiol. Biochem.* **45** (1), 88–107. https://doi.org/10.1159/000486225 (2018).
51. Soos, S., Balasko, M., Jech-Mihalffy, A., Szekely, M. & Petervari, E. Anorexic vs. metabolic effects of central leptin infusion in rats of various ages and nutritional states. *J. Mol. Neurosci.* **41** (1), 97–104. https://doi.org/10.1007/s12031-009-9294-4 (2010).
52. Sinitskaya, N. et al. Increasing the fat-to-carbohydrate ratio in a high-fat diet prevents the development of obesity but not a prediabetic state in rats. *Clin. Sci. (Lond).* **113** (10), 417–425. https://doi.org/10.1042/CS20070182 (2007).
53. Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates* (Academic, 2013).
54. Ujvári, B. et al. Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat. *J. Neuroinflammation*. **19** (1), 31. https://doi.org/10.1186/s12974-022-02399-w (2022).
55. Fuzesi, T., Wittmann, G., Liposits, Z., Lechan, R. M. & Fekete, C. Contribution of noradrenergic and adrenergic cell groups of the brainstem and agouti-related protein-synthesizing neurons of the arcuate nucleus to neuropeptide-y innervation of corticotropin-releasing hormone neurons in hypothalamic paraventricular nucleus of the rat. *Endocrinology*. **148**, 5442–5450. https://doi.org/10.1210/en.2007-0732 (2007).
56. Furedi, N. et al. Regulatory alterations of energy homeostasis in spontaneously hypertensive rats (SHR). *J. Mol. Neurosci.* **59** (4), 521–530. https://doi.org/10.1007/s12031-016-0771-2 (2016).

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Author contributions

All authors contributed to the study conception and design. Sz.E., M.B., F.P., D.K.K. performed the experiments. Material preparation, data collection and analysis were performed by N.F., B.G., V.K., G.B. and E.P. The first draft of the manuscript was written by Sz. E. and E. P. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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