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Neuropeptide Y Activity in the Nucleus Accumbens Modulates Feeding Behavior and Neuronal Activity

José K. van den Heuvel, Kara Furman, Myrtille C.R. Gumbs, Leslie Eggels, Darren M. Opland, Benjamin B. Land, Sharon M. Kolk, Nandakumar Narayanan, Eric Fliers, Andries Kalsbeek, Ralph J. DiLeone, and Susanne E. la Fleur

Department of Endocrinology and Metabolism (JKvdH, MCRG, LE, EF, AK, SEIF), Academic Medical Center, University of Amsterdam; and Hypothalamic Integration Mechanisms (AK), Netherlands Institute for Neuroscience, Amsterdam, Netherlands; Division of Molecular Psychiatry (KF, DMO, BBL, RJD), Yale University School of Medicine, New Haven, Connecticut; Department of Neurology (NN), Carver College of Medicine, University of Iowa, Iowa City, Iowa; and Department of Molecular Animal Physiology (SMK), Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, The Netherlands

Abstract

Background—Neuropeptide Y (NPY) is a hypothalamic neuropeptide that plays a prominent role in feeding and energy homeostasis. Expression of the NPY Y1 receptor (Y1R) is highly concentrated in the nucleus accumbens (Acb), a region important in the regulation of palatable feeding. In this study, we performed a number of experiments to investigate the actions of NPY in the Acb.

Methods—First, we determined caloric intake and food choice after bilateral administration of NPY in the Acb in rats on a free-choice diet of saturated fat, 30% sucrose solution, and standard chow and whether this was mediated by the Y1R. Second, we measured the effect of intra-Acb NPY on neuronal activity using in vivo electrophysiology. Third, we examined co-localization of Y1R with enkephalin and dynorphin neurons and the effect of NPY on preproenkephalin messenger RNA levels in the striatum using fluorescent and radioactive in situ hybridization. Finally, using retrograde tracing, we examined whether NPY neurons in the arcuate nucleus projected to the Acb.

Results—In rats on the free-choice, high-fat, high-sugar diet, intra-Acb NPY increased intake of fat, but not sugar or chow, and this was mediated by the Y1R. Intra-Acb NPY reduced neuronal firing, as well as preproenkephalin messenger RNA expression in the striatum. Moreover, Acb enkephalin neurons expressed Y1R and arcuate nucleus NPY neurons projected to the Acb.

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Address correspondence to José K. van den Heuvel, MSc, University of Amsterdam, Department of Endocrinology and Metabolism, Academic Medical Center, Meibergdreef 9, F2-154, Amsterdam-Zuidoost 1105 AZ, The Netherlands; j.k.vandenheuvel@amc.uva.nl; josevdheuvel@gmail.com.

Authors KF and MCRG contributed equally to this work.

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Conclusions—NPY reduces neuronal firing in the Acb resulting in increased palatable food intake. Together, our neuroanatomical, pharmacologic, and neuronal activity data support a role and mechanism for intra-Acb NPY-induced fat intake.

Keywords

Accumbens; diet; enkephalin; feeding; neuropeptide; obesity

A major population of neuropeptide Y (NPY)-producing neurons is located in the arcuate nucleus (ARC) of the hypothalamus. Central administration of NPY increases feeding behavior in rodents (1–3) and NPY has been linked to the control of carbohydrate intake (4,5). However, we recently observed that when NPY is administered in the lateral ventricle of rats on a free-choice, high-fat, high-sugar (fCHFHS) diet, it was not sugar but rather saturated fat and chow consumption that increased (6). The link between NPY and carbohydrate intake has been previously studied using administration of NPY into specific hypothalamic regions (5,7). In contrast, we administered NPY into the lateral ventricle, providing contact with not only the hypothalamus but also the corticolimbic areas involved in reward and motivation, such as the nucleus accumbens (Acb), where NPY receptors are localized (8–10). It remains to be explored, however, whether direct action of NPY in the Acb regulates fat consumption.

Reduced neuronal activity in the Acb occurs during feeding, and intra-Acb administration of orexigenic compounds, such as melanin concentrating hormone and muscimol, inhibit Acb neuronal activity (11–14). However, it is not known whether NPY also affects neuronal activity in the Acb. Given that direct NPY administration into other brain areas, including the amygdala and ARC, reduces neuronal activity (15,16), we hypothesized that NPY reduces Acb neuronal activity and that this is associated with increased fat intake.

Opioids have an established role in reward behavior, and opioid-expressing neurons are located within the Acb. Of the opioid-expressing neurons, enkephalin (ENK) neurons are of specific interest as they express dopamine D2 receptors and have been linked to high-fat feeding, i.e., Acb enkephalin gene expression is affected by consumption of the highly palatable Ensure drink (17), which contains fat and sugar, and striatal D2 receptor availability is correlated with high-fat intake (18). Moreover, enkephalin binds to mu-opioid receptors and local Acb administration of the mu-opioid receptor agonist DAMGO specifically increases intake of high-fat foods (19). In turn, the DAMGO-induced increased intake of high-fat foods is NPY Y1 receptor (Y1R) dependent (20). Therefore, the effect of NPY on fat intake may involve changes in Acb enkephalin levels. Given these findings, we hypothesized that Y1Rs are localized on enkephalin neurons and that an intra-Acb NPY injection alters striatal enkephalin levels.

Finally, previous studies have revealed anatomic connections between the ARC and Acb (21,22), as well as the presence of NPY receptors in the Acb (8–10). While a recent study found α -melanocyte-stimulating hormone (α -MSH) projections from the ARC to Acb (23), it has not been determined whether NPY neurons in the ARC project to the Acb.

To examine the action of NPY in the Acb, we first assessed food intake and preproenkephalin (ppENK) messenger RNA (mRNA) responses to intra-Acb NPY injections in rats on the fCHFS diet. Second, we measured NPY's effects on neuronal activity using in vivo electrophysiology. Third, using fluorescent imaging and retrograde tracing, we determined whether Y1Rs are localized on enkephalin and dynorphin neurons and whether NPY neurons in the ARC project to the Acb. We found that intra-Acb NPY stimulates fat intake via the Y1R and is associated with reduced expression of ppENK mRNA and neuronal activity. Moreover, ENK neurons express Y1Rs and NPY neurons in the ARC project to the Acb. These neuroanatomical, pharmacologic, and neuronal activity data support a possible role and mechanism for intra-Acb NPY-induced fat intake.

Methods and Materials

Animals

For behavior and radioactive in situ hybridization, male adult Wistar rats (Charles River, Germany) weighing 270 g to 300 g were used. For electrophysiological experiments and fluorescent in situ hybridization, C57BL/6 mice (Jackson Labs, Bar Harbor, Maine) weighing 25 g to 30 g were used. All animal procedures were performed in accordance with the protocol approved by the Yale Institutional Animal Care and Use Committee and the Committee for Animal Experimentation of the Academic Medical Center of the University of Amsterdam.

Effect of Intra-accumbens NPY and Y1R Antagonist on Food Intake

Rats were housed in a temperature- (21°C to 23°C) and light-controlled room (lights on 0700–1900). One week after arrival, rats were implanted with two cannulae aimed bilaterally at the Acb shell as described in Supplement 1. One week after surgery rats ($n = 15$) were placed on an fCHFS diet and were able to choose from the following components: a dish of saturated fat (beef tallow [Ossewit/Blanc de Boeuf, Vandemoortele, Belgium), a bottle of 30% sugar water (mixed from commercial grade sugar and tap water), standard chow (Special Diet Services, Essex, United Kingdom), and tap water. After 1 week of fCHFS diet exposure, .6 µg NPY [minimum effective dose in previous experiments (24,25) and own observations] or vehicle (1 × phosphate buffered saline [PBS]) was administered at the beginning of the light phase (between 1000 and 1100) in a balanced design. NPY was obtained from Bachem, Germany (H6375) and dissolved in 1 × PBS. Before the start of the experiment, all food components (except water) were removed from the cage. Injector cannulae (33-gauge) (Plastics One, Bilaney Consultants GmbH, Düsseldorf, Germany), extending 1 mm below the guide cannulae, were inserted into the guide cannulae, and animals received bilateral infusions of .3 µL fluid per site at a rate of .15 µL/minute via a syringe infusion pump. Injections were confirmed by monitoring fluid movement in the tubing via a small air bubble. After completion of the injection, the injector was left in place for 1 minute to allow for diffusion. Upon completion of all infusions, food was returned to the cages and all individual food components were measured after 2, 5, and 24 hours, and caloric intake (kcal) for each individual food item and total caloric intake were calculated. Total caloric intake was defined as the sum of each individual food component for which the

caloric density was defined as follows: chow: 3.31 kcal/g; fat: 9 kcal/g; and sucrose solution: 1.2 kcal/g. The experiment was repeated 3 days later according to a crossover design.

At the end of the experiment, food was removed in the morning (and not returned) and rats received intra-Acb injection of vehicle or .6 μ g NPY. One hour later, rats were anesthetized and transcardially perfused with ice-cold saline followed by 4% paraformaldehyde and postfixed for 2 hours. Brains were washed in PBS, cryoprotected in 30% sucrose at 4°C overnight, and subsequently frozen on dry ice and stored at -80°C. Cryostat sections were cut at 35 μ m and mounted on Superfrost Plus slides (Fisher, Gerhard Menzel GmbH, Germany). Some slides were stained for Nissl with thionine and checked for cannula placement with inclusion criteria described below. Remaining slides were air-dried and stored at -80°C to be used for radioactive in situ hybridization. The procedure for radioactive in situ hybridization was performed as described previously (26) and described in Supplement 1.

In a separate experiment, we tested whether the Acb NPY Y1 receptor was involved in the intra-Acb NPY-induced increased fat intake by intra-Acb administration of the Y1R antagonist GR 231118 (synonym of LY1229U91, Sigma Aldrich, Zwijndrecht, The Netherlands) or vehicle (in a volume of .2 μ L) in the Acb 15 minutes before intra-Acb NPY in rats on the fHFHS diet ($n = 15$). These experiments were conducted according to the same protocol (for surgery and cannula placement verification) as described above. We only measured intake of chow, fat, and sugar at one time point, as NPY effects do not show beyond a few hours. The dose of antagonist used (.3 μ g) was chosen based on reports published elsewhere (27,28) and on a dose response in a few animals using .3, 1, and 3 μ g antagonist. Since 1 μ g and 3 μ g in the Acb decreased overall intake, we chose the .3 μ g dose, because it did not significantly affect feeding behavior (data not shown). At the end of the experiment, rats were killed by decapitation and brains were removed, frozen on dry ice, and stored at -80°C. Cryostat sections were stained for Nissl with thionine and checked for cannula placement. Data of rats were only included when we verified that they had unilateral or bilateral cannula placements in the Acb shell between bregma 1.0 and 2.20 (Paxinos and Watson 4th ed. 1998) (29,30). Based on these criteria, five animals were excluded from analysis from experiment 1, and one animal was excluded from experiment 2 (with Y1R antagonist). Furthermore, for experiment 2, data from three more animals were excluded from analysis due to loss of the cannula or sickness behavior after injection, which turned out to be associated with infection around the cannula.

Fluorescent In Situ Hybridization

Fluorescent in situ hybridization was performed as described previously (31) and in Supplement 1. Probes for double in situ hybridization were prepared using an in vitro transcription kit with digoxigenin-labeled uridine 58-triphosphate (Roche, Basel, Switzerland) for making the Y1R probe or fluorescein-labeled uridine 58-triphosphate (Molecular Probes, Eugene, Oregon) for making the ppENK or prodynorphin probe (information on probes is provided in Supplement 1).

In Vivo Electrophysiology

Four well-habituated C57BL/6 mice (25 g to 30 g) were implanted with a cannula (Plastics One) and arrays of microwire electrodes (Tucker-Davis Technologies, Alachua, Florida) using aseptic stereotaxic methods described in detail previously (12) and in Supplement 1. The arrays consisted of 16 Teflon-coated, 50 μm stainless steel wires arranged in an 8×2 configuration, with each electrode spaced by $\sim 250 \mu\text{m}$. The in vitro impedance of the electrodes was 100 k Ω to 300 k Ω . After a recovery period of 5 to 7 days, animals were acclimated to recording procedures (i.e., headsets and cables were attached to the implants) for 1 day. Drug infusions were performed as follows. Animals were lightly anesthetized with halothane via a nose cone during the experiment, and recording headstages were plugged in. After initial recording, data acquisition was paused and animals were infused with either artificial cerebrospinal fluid (aCSF) (sodium chloride 147 mmol/L, calcium chloride 1.3 mmol/L, magnesium chloride .9 mmol/L, potassium chloride 4.0 mmol/L) or NPY (.6 μg). Infusions were made through 33-gauge cannulae (Plastics One) that protruded .2 mm from the tip of the guide cannulae. Injectors were inserted into the guide cannulae and .3 μL of infusion fluid was delivered per site at a rate of .15 $\mu\text{L}/\text{minute}$ via a syringe infusion pump (KDS Scientific, Holliston, Massachusetts). Fluid was infused via .38-mm diameter polyethylene tubing that connected the injector to a 5 μL Hamilton syringe (Roche). Injections were confirmed by monitoring movement of fluid in the tubing via a small air bubble. After injection was complete, the injector was left in place for 2 minutes to allow for diffusion. Infusions took place between 1200 and 1400. Recording data were collected for 1 hour after infusions.

Neuronal ensemble recordings were made using a Many Neuron Acquisition Program (Tucker-Davis Technologies). Putative single neuron units were identified online using an oscilloscope and an audio monitor. The Tucker-Davis Technologies offline sorter was used to analyze the signals offline and to remove artifacts due to cable noise and behavioral devices (pump, click stimulus). Principal component analysis and waveform shape were used for spike sorting. Single units were identified as having 1) consistent waveform shape, 2) separable clusters in principal component analysis space, 3) average amplitude estimated at least three times larger than background activity, and 4) a consistent refractory period of at least 2 milliseconds in interspike interval histograms. Those units identified online as potential single units that did not meet these criteria offline were not included in this analysis. Datasets were previewed using OpenSorter (Tucker-Davis Technologies) and subsequently analyzed using custom routines for MATLAB (MathWorks, Natick, Massachusetts). For each well-isolated neuron, postinfusion firing rates were normalized to mean preinfusion firing rates (in the 10 minutes immediately preceding drug infusion) and binned (60-second bins). Activity was then compared between neurons recorded in NPY and aCSF conditions.

At the end of the experiment, mice were perfused transcardially with ice-cold saline followed by 4% paraformaldehyde. After fixation, brains were cryoprotected in 30% sucrose at 4°C overnight. Microtome sections were cut at 40 μm along the horizontal axis and mounted on Superfrost Plus slides (Fisher). Electrode and cannulae placement in horizontal

sections were verified microscopically using the mouse brain atlas from Paxinos and Franklin (2004).

Cholera Toxin B Tracing

One week after arrival, six male Wistar rats (Charles River) weighing 270 g to 300 g received two cannulae: one aimed at the right Acb shell for infusion of the cholera toxin B (CTB) conjugated to alexa-555 (C-22843, Invitrogen, Bleiswijk, The Netherlands) tracer and one cannula in the left lateral ventricle for the infusion of colchicine to block neuronal transport (C9754, Sigma-Aldrich, Zwijndrecht, The Netherlands). Surgical procedures were the same as for the rat study described above. For the lateral ventricle, a permanent 22-gauge stainless steel guide cannula was used with coordinates .8 mm posterior from bregma, 1.4 mm lateral from the midline, and 4.5 mm below the surface of the brain. In contrast to CTB, fluorophore-labeled CTB cannot be applied by iontophoresis, so we used pressure injection (21). Rats were injected unilaterally with 100 nL to 150 nL 1% CTB into the Acb shell. The injection needle was left in place and fixed with dental cement to the skull to minimize leakage from the tract (21). Twelve days later, rats were deeply anesthetized and 100 µg colchicine in 5 µL PBS was injected in the lateral ventricle. Twenty-four to 36 hours later, rats were transcardially perfused with saline, followed by a solution of 4% paraformaldehyde in .1 mol/L PBS (pH 7.4) at 4°C. Brains were removed and kept in a 30% sucrose solution dissolved in PBS for 2 hours. Subsequently, the brains were washed briefly with PBS, frozen on dry ice, and stored at -80°C until further processing for fluorescent immunohistochemistry. The procedure for the fluorescent immunohistochemistry was performed as described previously (32) and in Supplement 1.

Statistical Analysis

For the effects of NPY on food intake and on enkephalin mRNA, t tests were performed. The data on NPY in combination with the Y1R antagonist were tested with a two-way repeated-measures analysis of variance (ANOVA) (for paired comparison) and were followed with Sidak's multiple comparisons tests. Data obtained with electrophysiology were analyzed with a repeated-measures ANOVA, followed by Tukey's post hoc tests where appropriate. For all analyses, significance was assigned at the $p < .05$ level. All data are presented as mean \pm SEM.

Results

Effect of NPY and Y1R Antagonist in Nucleus Accumbens on Food Intake

Local administration of NPY significantly increased fat intake after 2 hours (Figure 1A) but not after 5 hours and 24 hours (Figure S1 in Supplement 1). Intra-Acb NPY did not affect intake of the chow or sugar component after 2 hours (Figure 1A) or after 5 hours and 24 hours (Figure S1 in Supplement 1). Subsequently, the effect of pretreatment with the selective Y1R antagonist GR 231118 on NPY-induced fat intake was tested. Again, when infused in the Acb, NPY specifically increased fat intake (Figure S2 in Supplement 1). Interestingly, when cannulae were placed bilaterally in the shell of the Acb (which was the case in 5 out of 11 animals), pretreatment with Y1R antagonist completely prevented the NPY-induced increase in fat intake (interaction pretreatment with Y1 antagonist \times NPY,

$F_{1,20} = 6.3$; $p = .02$); a trend for NPY effect ($F_{1,20} = 3.5$; $p = .08$); and no effect of the Y1 antagonist ($F_{1,20} = 1.5$; $p = .24$) (Figure 1B).

NPY Reduces Cell Firing in the Nucleus Accumbens In Vivo

To evaluate the effects of NPY on neuronal activity in vivo, neuronal firing data were collected during intra-Acb infusions of aCSF or NPY with microwire recording electrodes implanted in the Acb (Figure 2A,B). Multiunit recordings performed under anesthesia revealed a significant reduction in firing rates of Acb neurons treated with .6 μg NPY ($n = 29$) and a significant interaction between time and treatment, $p = .0010$, two-way ANOVA $F_{39,1482} = 1.874$ (Figure 2C,D). Firing in 11/29 (37.9%) units was suppressed 50% below from baseline. Another 9/29 (31.0%) units were suppressed 25% below from baseline. The remaining 9 units were either unchanged (6 [20.7%] of 29) or increased (3 [10.3%] of 29) relative to preinfusion firing rates. This effect lasted 25 minutes, starting within 5 minutes after infusion (Figure 2C).

NPY Y1R Co-localizes with Enkephalin and Dynorphin Positive Neurons in Nucleus Accumbens

Since NPY receptors are present in the Acb (10), we investigated whether Y1R co-localizes with enkephalin and dynorphin neurons by performing double-labeled fluorescent in situ hybridization. Co-localization of Y1R on enkephalin and dynorphin neurons was found throughout the striatum and Acb; about 59% of Y1R co-localized with enkephalin neurons (Figure 3A) and about 18% with dynorphin neurons (Figure S3 in Supplement 1) as revealed by confocal analysis.

NPY Administration in the Nucleus Accumbens Decreases ppENK mRNA Expression

Striatal enkephalin, as opposed to dynorphin, has been described to be affected by changes in energy balance (33). Additionally, we found that Y1R is mainly located on enkephalin neurons. Therefore, we investigated the effect of NPY on enkephalin mRNA levels. We quantitatively measured ppENK mRNA levels using radioactive in situ hybridization and found that 1 hour after intra-Acb NPY administration, ppENK mRNA expression was significantly downregulated in both the ventral striatum (Acb) as well as the dorsal striatum (Figure 3B) ($p < .05$).

Subset of Arcuate NPY Neurons Project to Nucleus Accumbens

To investigate the origin of NPY neurons projecting to the Acb, the CTB tracer was administered in the Acb and co-localization with NPY antibody was determined. The tracer was mostly concentrated at the border of the medial shell and core, positioned dorsomedially to the anterior commissure (Figure 4A). Anterograde CTB tracing was found in the medial part of the subcommissural ventral pallidum and lateral hypothalamus confirming previous findings (34–36). In this study, our main focus was to investigate afferent projections to the Acb and consequently to determine which of these projections co-label with NPY. Retrograde transport of CTB tracer was examined by serial sectioning and immunostaining in the neocortex, thalamus, hypothalamus, amygdaloid nuclei, ventral pallidum, and subiculum.

Consistent with previous studies, CTB staining was found in labeled perikarya in the agranular insular cortex, perirhinal cortex, midline thalamic nuclei, and (baso)lateral amygdala (22,35,36). However, in none of these areas did CTB co-localize with NPY staining.

The primary site for synthesis of NPY peptide is the ARC. Consistent with others (21,22), we found CTB staining in the ARC. CTB labeling was present in the ventral medial and ventral lateral parts of the ARC. In the ventral medial ARC, the majority of CTB cells were dually immunostained with NPY (Figure 4B), indicating NPY positive cells projecting from the ARC to Acb. This finding was confirmed in three successfully injected cases. CTB cells in the lateral portion of the ARC, which did not co-localize with NPY, may be α -MSH neurons, as melanocortinergic projections from the ARC to Acb have been shown previously (23).

Discussion

In the present study, we used a combination of behavioral, neuroanatomical, and in vivo electrophysiological measures to investigate the effect of NPY on Acb neuronal activity, neuropeptide expression, and behavioral output. We demonstrated that NPY administration in the Acb in rats on a fCHFHS diet increased fat intake specifically, and this effect was attenuated by pretreatment with a Y1R antagonist in the Acb shell. Furthermore, we identified NPY Y1 receptors on ENK neurons in the Acb and found that NPY administration lowered Acb neuronal activity and levels of striatal ppENK mRNA. Finally, we showed that a subset of ARC NPY neurons project to the Acb. Together with our earlier findings that a fCHFHS diet increased NPY mRNA in the ARC and increased sensitivity for NPY (37,38), these findings provide evidence for a role of NPY in the intake of palatable foods, possibly via inhibition of D2-enkephalin neurons. Moreover, the increased levels of NPY mRNA in the ARC observed in rats on a fCHFHS diet may, via projections to the Acb, be related to the increased motivation and active lever pressing as well as the persistent hyperphagia observed in these rats (37,39).

Consistent with others, we found that intra-Acb NPY did not affect chow (4,25) and sucrose (25) intake. Interestingly, a recent study showed that intra-Acb NPY increased intake of sucrose pellets (40), which implies that intra-Acb NPY may increase sucrose intake in the absence of fat or that the effect is specific to solid and not liquid sugar. NPY has been associated with carbohydrate intake in a number of studies (4,5,41), yet NPY is often administered in the third ventricle or directly into hypothalamic areas. In this study, NPY was directed to the Acb and specifically increased fat intake, suggesting that the effect of NPY on food component consumption may be dependent on the specific brain site. Basal caloric intake in rats on the fCHFHS diet comprises 50% from chow, 30% from sugar, and 20% from fat. Because fat is not the main preferred component under baseline conditions with this diet, we infer that NPY-induced fat intake is not confounded by a pre-existing baseline fat preference.

Our findings indicate that NPY inhibits neuronal activity in the Acb. To our knowledge, this is the first study investigating the effects of NPY on Acb neuronal activity. The inhibitory

effects in the Acb are inconsistent with NPY's inhibitory effects in other brain areas, such as the amygdala and ARC (15,16), and coincide with the downregulation of ppENK mRNA expression upon intra-Acb NPY administration, another novel finding in this study. This may suggest that NPY's feeding effects in the Acb may occur via downregulation of D2-containing ENK neurons. The ability of NPY to reduce neuronal firing in the Acb is consistent with a general model from other pharmacologic and electrophysiologic studies in which reduced Acb neuronal firing stimulates food intake (12,14). Future studies are needed to determine the precise mechanism underlying NPY's inhibitory actions in the Acb.

Mu-opioid receptor agonists (such as DAMGO) elicit robust feeding responses; however, administration of different doses of Met-enkephalin or a synthetic and protease-resistant analog of enkephalin ([D-Ala2] Met-enkephalin) in different Acb regions did not produce any significant effects on food intake (42). The majority of enkephalin neurons co-express gamma-aminobutyric acid (GABA) (43). Therefore, the NPY-mediated downregulation of ppENK neurons may also represent a reduction of GABAergic output. Reduced Acb GABAergic output has consistently been shown to elicit intense feeding in satiated rats and to increase intake of palatable foods (11,13,14,44,45). This reduced Acb GABAergic output likely affects food intake via the lateral hypothalamus (LH) and medial ventral pallidum (46). The downstream outputs from the LH involve autonomic structures and structures that directly control brainstem pattern generators for the motor actions of eating (47). Therefore, we speculate that the downstream mechanisms of the inhibitory effects of NPY in the Acb (leading to the increase in fat intake) involve reduced GABAergic output to the LH via the medial ventral pallidum.

We demonstrated CTB tracing and NPY staining in brain areas consistent with others (22,48). However, co-localization was only found in the ARC, suggesting that NPY neurons projecting to the Acb predominantly originate from the ARC. Although it remains to be determined whether ARC-derived NPY is the principal mediator in palatable feeding regulation when rats are on a fCHFS diet, the NPY-ergic ARC projections to the Acb supplement findings describing α -MSH projections from the ARC to the Acb (23) and suggest that afferent signals from ARC to Acb provide input about the general metabolic state of the body. Receptors of nearly all known metabolic hormones, such as leptin, insulin, glucocorticoid, and ghrelin, are present on ARC NPY neurons. It remains to be explored whether the NPY neurons that project to the Acb are also controlled by these peripheral signals.

In conclusion, our data indicate a role for NPY in the Acb in the intake of palatable food, which is likely mediated by inhibition of enkephalin neurons, probably leading to decreased GABAergic output. Future studies are needed to establish a direct role of GABAergic enkephalin neurons in NPY's effect on fat intake. Our findings confirm and extend research suggesting a role for NPY in the feeding-related neural circuit controlled by the Acb (20,49,50). The feeding effects of the hypothalamic peptide NPY in the Acb further links the homeostatic and hedonic feeding circuits in the control and regulation of palatable feeding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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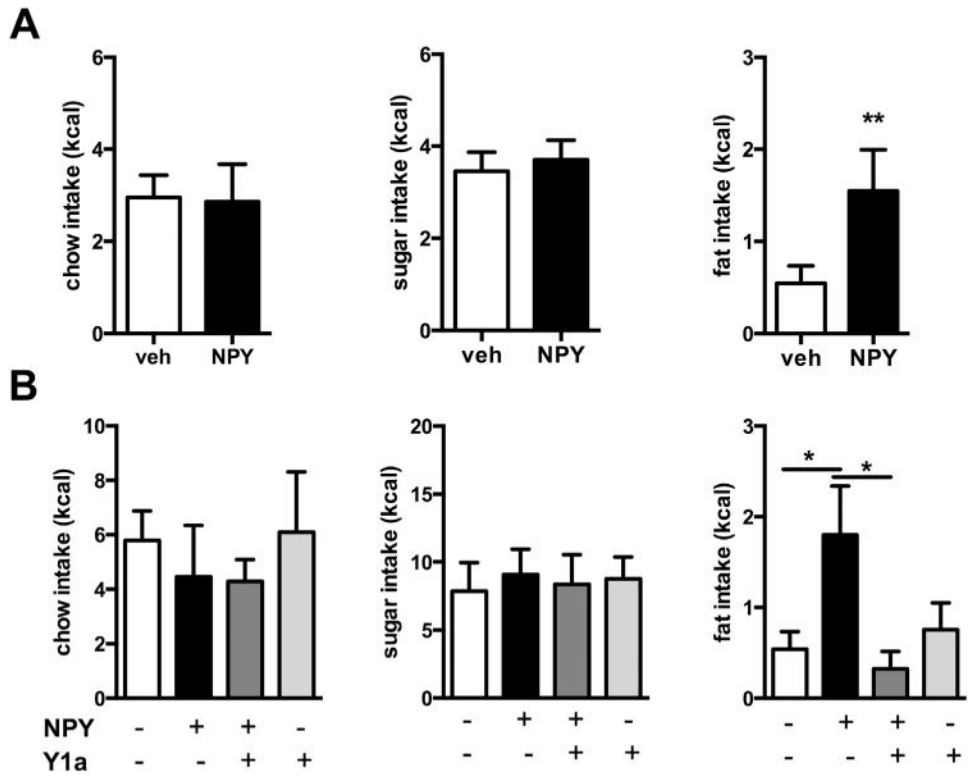


Figure 1. Effect of intra-nucleus accumbens (Acb) neuropeptide Y (NPY) in rats on intake of chow, sugar, and fat components of the free-choice, high-fat, high-sugar diet. **(A)** NPY in the Acb significantly increased fat intake and not sugar or chow intake; total intake tended to be increased (vehicle [veh] $6.5 \pm .8$ vs. NPY 8.7 ± 1.1 ; $p = .1$) ($n = 10$). **(B)** The increase in fat intake was prevented by pretreatment with Y1 receptor antagonist (Y1a) GR 231118, injected bilaterally into the shell of Acb, whereas the Y1 receptor antagonist did not affect fat intake (or chow and sugar) alone ($n = 5$). Data are presented as mean \pm SEM. A significant effect is depicted by $*p < .05$ and $**p < .01$.

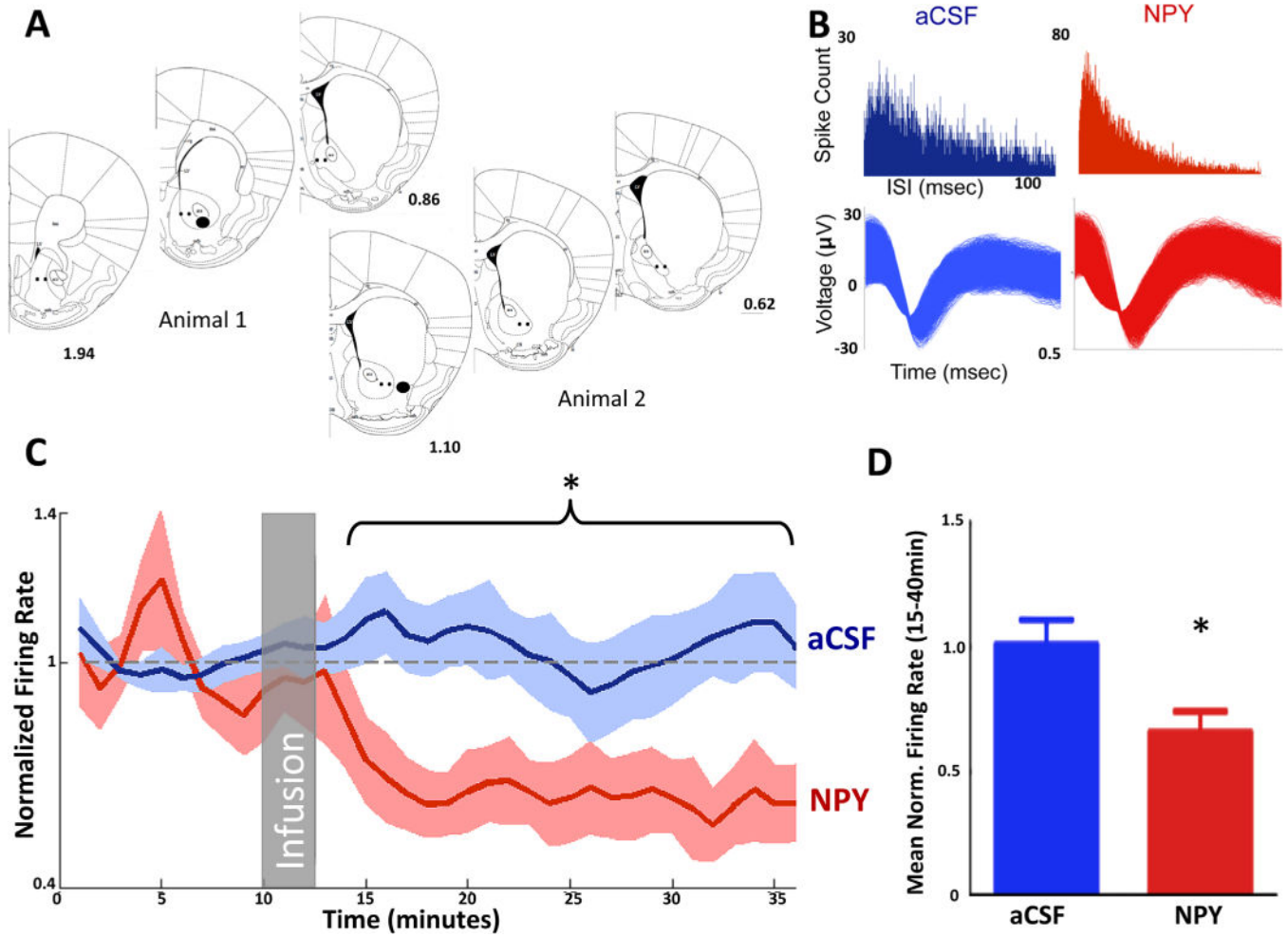


Figure 2.

Neuropeptide Y (NPY) in the nucleus accumbens decreases neuronal activity in vivo. **(A)** Location of electrodes (black spots) and cannulae (filled black circles) projected on a mouse brain atlas section (51). **(B)** Interspike interval (ISI) histograms and waveforms from a single unit in artificial cerebrospinal fluid (aCSF) infusion sessions (left) and the same neuron in NPY infusion sessions (right). **(C, D)** Average neuronal activity for NPY sessions and aCSF. Gray box represents time of infusion. NPY significantly reduces firing rate from 15 minutes to 40 minutes. * $p < .05$. Norm., normalized.

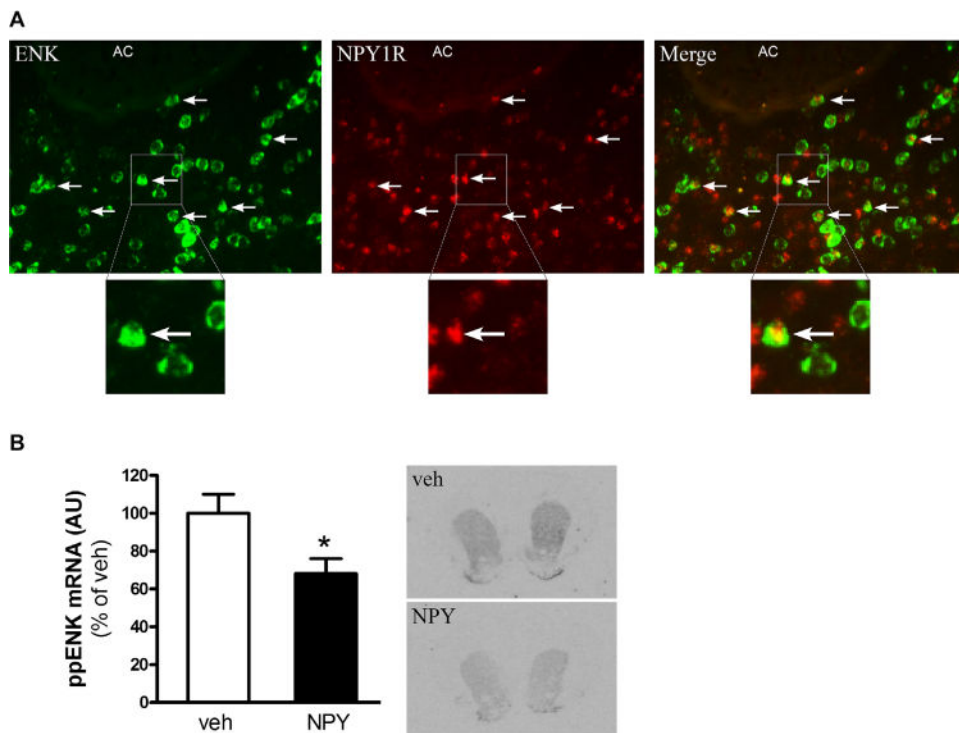


Figure 3. Neuropeptide Y (NPY) Y1 receptor (NPY1R) is expressed on enkephalin (ENK) neurons in the nucleus accumbens (Acb) and intra-Acb NPY downregulates preproenkephalin (ppENK) messenger RNA (mRNA) levels. **(A)** Expression pattern of Y1R and ENK in the Acb. Horizontal arrows indicate co-localization. **(B)** ppENK gene expression levels in the ventral striatum and representative images after intra-Acb injection of NPY. Values are means \pm SEM of seven to nine rats per group. * $p < .05$ NPY vs. vehicle (veh). AC, anterior commissure.

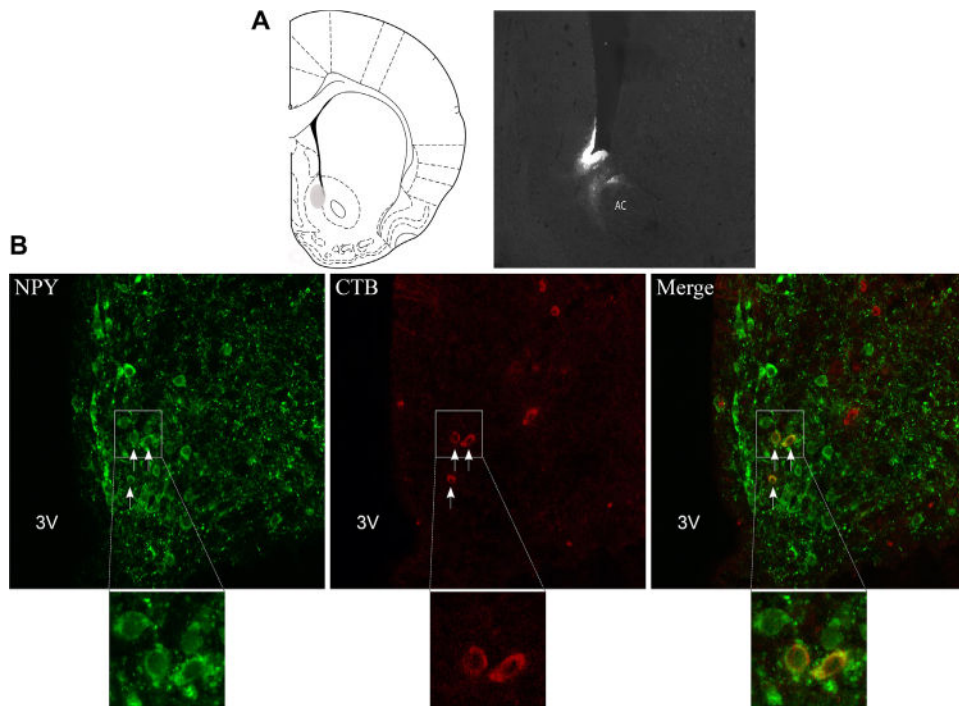


Figure 4. Neuropeptide Y (NPY)-positive neurons in the arcuate nucleus project to the nucleus accumbens. (A) Representative image for cholera toxin B (CTB) injection in the nucleus accumbens. (B) Confocal microscopy shows co-localization of NPY and CTB in the arcuate nucleus of rats. Arrows indicate co-localization. AC, anterior commissure; 3V, third ventricle.