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Overexpression of neuropeptide Y in the dorsomedial hypothalamus increases trial initiation but does not significantly alter concentration-dependent licking to sucrose in a brief-access taste test

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HIGHLIGHTS

- Rats with adeno-associated virus mediated overexpression of NPY (AAVNPY) in the DMH.
- Sucrose-concentration series in a brief-access taste test.
- AAVNPY rats initiated more trials than AAVGFP controls.
- Consistent with hypothesis that main feeding effects are on appetitive function.
- No significant group differences in lick response across the sucrose concentrations.

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ABSTRACT

Evidence in the literature raises the possibility that alterations in neuropeptide Y (NPY) in the dorsomedial hypothalamus (DMH) may contribute to hyperphagia leading to body weight gain. Previously, we have shown that compared to AAVGFP controls, adeno-associated virus (AAV)-mediated overexpression of NPY in the DMH of lean rats resulted in significantly higher body weight gain that was attributed to increased food intake, and this was further exacerbated by a high-fat diet. Here, we tested AAVNPY and AAVGFP control rats in a brief-access taste procedure (10-s trials, 30-min sessions) to an array of sucrose concentrations under *ad libitum* and partial food and water access conditions. The test allows for some segregation of the behavioral components by providing a measure of trial initiation (appetitive) and unconditioned licks at each concentration (consummatory). Consistent with previous findings suggesting that NPY has a primary effect on appetitive function, overexpression of DMH NPY did not significantly alter concentration-dependent licking response to sucrose but when tested in a non-restricted food and water schedule, AAVNPY rats initiated significantly more sucrose trials compared to AAVGFP controls in a brief-access taste test.

1. Introduction

Following its discovery [37], neuropeptide Y (NPY) has been shown to be widely distributed in the mammalian central [1] and peripheral nervous systems [16,25]. Central administration of NPY in rats increases food intake [10,23,36] and, with long-term administration, induces increases in body weight and body fat [35,43]. Consistent with evidence pointing to the importance of hypothalamic peptide systems in energy balance mechanisms, NPY has been found throughout the hypothalamus [9,16] including localization in the arcuate nucleus and dorsomedial hypothalamus (DMH) [8,9,16,41]. Running wheel access and food restriction [24], as well as running wheel access alone [20], have been shown to induce elevated NPY protein or mRNA levels in the DMH. Furthermore, elevated expression of NPY in the DMH has been observed in a number of obesity rodent models, including in homozygous melanocortin 4 receptor (MC4-R) knockout mice [21], obese tubby mice [18], diet-induced obese mice [17] and obese brown adipose tissue-deficient (uncoupling protein-promoter-drive diphtheria toxin A) mice [40]. Increased expression of DMH NPY has also been found in young pre-obese Otsuka Long–Evans Tokushima Fatty (OLETF) rats and OLETF rats that have been pair-fed to match food intake of lean LETO controls [7], thus raising the possibility that alterations in NPY in the DMH precedes the hyperphagia that leads to weight gain, in at least some of these obesity models.

More recently, our group has used the adeno-associated virus (AAV) system to either increase or decrease *Npy* gene expression in the DMH of rats. Compared to AAVGFP controls, lean rats with overexpression of DMH NPY displayed significantly higher body weight gain, which appeared to be attributed by an increase in chow intake and was further exacerbated by the presentation of a high-fat diet [42]. In contrast,

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knockdown of NPY in the DMH *via* AAV-mediated RNAi in OLETF rats, significantly reduced daily food intake, body weight gain, lowered hyperglycemia and decreased fat accumulation compared to control OLETF rats, to a degree similar to that of the lean LETO group [42].

Ingestive behavior can be thought of as consisting of an appetitive component which involves behavior that brings the animal to the stimulus and a consummatory component which describes behavior following stimulus contact with the oral cavity (see [12]). Based on previous findings in the literature, it has been suggested that the main feeding effects of NPY are on increases of appetitive but not consummatory behavior. A number of studies have shown that intracerebroventricular (ICV) administration of NPY increases intake of a sucrose solution when presented in a one-bottle test which involves both appetitive and consummatory behavior, but not when the solution is infused intraorally, a measure which focuses more on the consummatory component of ingestion [3,4,29,30].

In contrast, prior behavioral training and stimulus exposure have been shown to interact with the effects of NPY so that, for example, ICV NPY administration can elicit increases in intraoral sucrose intake [6]. Furthermore, ICV NPY administration has been shown to increase the size of meal, which can be regarded as a measure of consummatory behavior with little or no significant change in meal frequency, which can be thought of as a measure of appetitive behavior [22,27]. Meal pattern analysis revealed that the decrease in chow intake observed in OLETF rats with knockdown of NPY expression in the DMH, was primarily attributed to a decrease in meal size compared to OLETF controls [42]. Consistent with these results, NPY knockdown in the DMH of intact rats produces a nocturnal and meal size-specific feeding effect [42]. Licking microstructure analysis has revealed an increase in meal frequency for water, saccharin and sucrose in response to ICV NPY in rats. However, for sucrose, NPY also elicited an increase in meal size [5]. Collectively, these findings suggest that in addition to increasing appetitive feeding, under certain test conditions (*e.g.* prior training, stimulus exposure and caloric content of stimulus), NPY can also increase consummatory components of behavior.

In the current study, unconditioned licking responses to a sucrose concentration array are compared between rats with AAV-mediated overexpression of *Npy* in the DMH (AAVNPY) and their AAVGFP controls. The brief-access taste procedure allows for some segregation between appetitive (spout approach measured as number of trials initiated) and consummatory (lick responses across the concentration range within each 10-s trial) behaviors. The two groups were tested in non-deprived (*ad libitum* access to chow and water) and partial food-and-water restricted (~10 g chow, ~20 ml water) conditions. If DMH NPY is primarily involved in behaviors that bring the animal to the stimulus, we would expect AAVNPY animals to initiate significantly more trials compared to their AAVGFP controls, with little or no group difference in licking across the sucrose concentration range. Alternatively, if the ingestive effects of NPY are *via* orosensory alterations, this may also impact hedonic responses to oral stimuli thus resulting in group differences in concentration-dependent lick responses.

2. Materials and methods

2.1. Subjects

Sixteen male Sprague–Dawley rats (Charles River Breeders) weighing 351.4 ± 3.4 g on the first day of behavioral training were individually housed in hanging wire mesh cages in a room where humidity, temperature and a 12 h–12 h light–dark cycle (lights on at 7:00 am) were automatically controlled. The animals were provided *ad libitum* chow (Prolab RMH 1000) and distilled water, except where noted. Behavioral testing sessions were conducted during the light cycle. All procedures were approved by the Institutional Animal Care and Use Committee at The Johns Hopkins University.

Behavioral testing began after at least 7 days acclimation in the lab environment. During behavioral training, the rats were placed on a water-restricted schedule. Water access was removed from the home cages no more than 23 h before testing and water was available only during the daily training sessions. *Ad libitum* access to water resumed in the home cages after the last training session. A partial food and water restriction condition to encourage sampling without imposing a 24-h deprivation schedule and to provide a condition to compare responses during different states of deprivation was included. Rats were presented ~10 g of chow and ~20 ml of water in the home cages for approximately 23 h before testing as adapted from studies in mice [15] and since used to test rats (*e.g.* [28]). Body weight was measured every day during water or partial food and water restriction conditions and did not fall below 85% of weight during *ad libitum* feeding.

2.2. Taste stimuli

All solutions were prepared daily with distilled water and presented at room temperature. Six concentrations of sucrose (0.01, 0.03, 0.06, 0.1, 0.3, and 1.0 M; Sigma Aldrich, St. Louis MO) were used.

2.3. Behavioral procedure

Training and testing were conducted in a lickometer (Davis MS-160, DiLog Instruments, Tallahassee FL) as previous described elsewhere (*e.g.* [15,31]). The rat was placed in the testing chamber of the apparatus and given access to a single spout positioned approximately 5 mm behind a slot. The spout was connected to a glass container holding a taste stimulus. A small fan was positioned above the chamber wall slot to direct a current of air past the drinking spout and to minimize potential olfactory cues from the stimulus. The rat initiated a trial by licking the spout. At the end of each trial (10 s), the shutter closed. During each 8-s intertrial interval, a motorized block moved to change the tube presentation and the shutter reopened for the next trial. Concentrations were presented in randomized blocks (without replacement). The animals were able to initiate as many trials as possible during the 30-min sessions.

2.4. Behavioral training and testing

The animals were trained and tested at the start of the experiment and ~4 weeks after bilateral AAV injection into the DMH (post-surgical testing). For the first 5 days of behavioral training and testing, the animals were placed on a ~23 h water restriction schedule in which water was available only during the daily 30-min sessions. On days 1 and 2, rats were presented with a stationary spout of water for 30 min. Total number of licks and inter-lick-interval were measured. On days 3 and 4, 7 tubes of water were prepared and presented one at a time in 10-s trials across 30-min sessions. On day 5, the 7 tubes were prepared with varying sucrose concentrations and presented in a similar manner.

After two days of rehydration, the animals were presented with the same array of sucrose concentrations for four consecutive days alternating testing conditions between non-restricted (*ad libitum* access to food and water) and partial-food-and-water states, as outlined in Table 1.

Table 1
Experimental design.

Condition	Days	Stimulus
Water restricted	2	Stationary water
Water restricted	2	Multiple presentations of water
Water restricted	1	Multiple concentrations of sucrose
Two days hydration, no testing		
Ad lib water + food	1	Multiple concentrations of sucrose
Partial food and water restriction	1	Multiple concentrations of sucrose
Ad lib water + food	1	Multiple concentrations of sucrose
Partial food and water restriction	1	Multiple concentrations of sucrose