The role of hypothalamic peptides in consummatory feeding behavior

Kelli Carter and Erin Lester
Honors College Thesis
Spring 2004

Dr. Sue Ritter
VCAPP, Programs in Neuroscience
Washington State University

Acknowledgements: We thank Sue Ritter for her guidance on this project.

Honors Thesis
*-*-*-*-*-*-*-*-*-*-*-*-*-*-*-*-*-*-*
PASS WITH DISTINCTION
TO THE UNIVERSITY HONORS COLLEGE:

As thesis advisor for Kelli Carter and Erin Lester

I have read this paper and find it satisfactory.

_Thesis Advisor_

3/26/04

Date
Précis

Many chemicals have been identified in the brain as being involved in the initiation of food intake of which there are two components: the appetitive component and the consummatory component. The appetitive component involves behaviors such as searching for and obtaining food. The consummatory component involves behaviors such as chewing, licking, and swallowing food once it is in the mouth. We proposed that the brain neurochemicals norepinephrine (NE), neuropeptide Y (NPY), and Agouti-related peptide (AgRP) would increase consummatory feeding behavior following direct injection into the area of brain tissue known as the hypothalamus.

NE, NPY, and AgRP have consistently been shown to increase the appetitive feeding behavior in rats, through many different feeding situations such as the eating of food pellets, bottle-feeding, and lever pressing for food pellets. However in previous studies, NPY has been shown to decrease the consummatory feeding behavior of rats. These findings are not consistent with the role NPY plays in the feeding pathway, nor are they consistent with other findings related to other such notorious feed enhancing neural chemicals as NE and AgRP.

If our data supports this hypothesis, we would better understand the neural circuitry governing feeding behavior in general and this understanding could potentially help us to find new treatments for diseases that are associated with the control of food intake, such as obesity and diabetes.

In order to address this hypothesis, we implanted small metal tubes (cannulas) directly into the brain area of interest so that these chemicals could be directly administered. Then we implanted small metal tubes (fistulas) into the cheeks of the rats.
so that we could directly infuse food into the mouth thereby by-passing the appetitive component of feeding and allowing us to directly measure consummatory feeding responses. These methods were conventional for the field of neuroscience. While the design of the cheek fistula for infusing food into the mouth was innovative for our field, it is standard procedure in our laboratory and is an improvement on the conventional fistula designs currently used by other investigators. The only problems with our procedure were problems that are normally encountered when working with live animals, i.e. illness and behavioral discrepancies. The only way to correct for these problems is to obtain replacement animals and test multiple experimental groups of animals.

Our findings showed that the chemicals injected (NE, NPY, and AgRP) all significantly increased consummatory food intake. This means that there must be some communication between the hypothalamus and the hindbrain, where reflex control sites governing consummatory responses are located. These results also mean that appetitive feeding behaviors are integrated with consummatory feeding behaviors. Our findings elucidate the neural circuitry and neurochemcial mediation of ingestive behaviors and we are one step closer to better understanding diseases of food intake control, such as diabetes and obesity.

Our results contradict the findings from similar studies, but our results are more compatible with the knowledge concerning food intake control in our field. The next step in this area of research would be to inject the same chemicals (NE, NPY and AgRP) directly into the hindbrain and test the effects on consummatory feeding there.
# Table of Contents

List of Figures ................................................................. 6  
Abstract .................................................................................. 7  
Introduction ............................................................................. 8  
Materials and Methods ............................................................ 10  
Results .................................................................................... 17  
Discussion ............................................................................... 21  
Conclusion ............................................................................... 24  
References ............................................................................... 25
List of Figures

Figure 1. Depicting the design and implementation of the stainless steel cheek fistula used for intraoral delivery of liquid food.................................12

Figure 2. Verification of Cannula Placements.............................................18

Figure 3. Cumulative intraoral intake following norepinephrine injection..19

Figure 4. Cumulative intraoral intake following neuropeptide Y injection........................................................................................................19

Figure 5. Cumulative intraoral intake following Agouti-related protein injection.....................................................................................................20
Abstract

Carter, K. and Lester, E. The role of hypothalamic peptides in consummatory feeding behavior. There are two components of feeding behavior that are highly regulated, an appetitive component, which involves the motivated acquisition of food, and the consummatory component, which involves the chewing and swallowing of food once it is in the mouth. Norepinephrine (NE), neuropeptide Y (NPY), and Agouti-gene-related protein (AgRP) were administered intracranially into the paraventricular nucleus of the hypothalamus (PVN) to examine the effects of these peptides on consummatory feeding behavior in the rat. Milk was subsequently infused intraorally through a chronic cheek fistula until rejected in order to measure the consummatory food intake. It was found that injection of NE, NPY, and AgRP all significantly increased intraoral intake. In conjunction with prior research, these findings suggest that NE, NPY, and AgRP all increase feeding. These results show that these peptides increase both the appetitive and consummatory feeding behaviors in response to intracranial injection into the PVN. Because all NE innervation of the PVN and approximately 50% of the NPY innervation arises from hindbrain NE and NPY neurons, the findings from this study are in agreement with other studies suggesting that the circuitry for consummatory ingestive responses is controlled in part by hindbrain mechanisms.

Keywords: norepinephrine, neuropeptide Y, agouti-related protein, food intake, consummatory feeding
Introduction

The behavior of food ingestion is highly regulated. There are two components of feeding behavior, an appetitive component and a consummatory component. The appetitive response involves the motivated acquisition of food, while the consummatory response involves the chewing and swallowing of food once it is in the mouth [1, 2, 3].

One of the neurotransmitters thought to have a primary role in energy homeostasis is norepinephrine (NE). NE has also been shown to elicit feeding behavior after injection into the hypothalamus [4]. After injection into the paraventricular nucleus of the hypothalamus (PVN), a variety of effects including increases in feeding behavior and corticosterone release, hypoglycemia, and alteration of metabolism occur [4].

Neuropeptide Y (NPY) is another neurotransmitter that has been found to be a very potent stimulator of food intake. Injection of NPY into the PVN has been shown to stimulate appetitive feeding, regulate energy metabolism, change body temperature, and increase insulin levels [5]. In addition, the orexigenic effects of NPY have a significantly longer duration than that of the catecholamines such as NE and epinephrine (E) [4].

A third neuropeptide that has been shown to have orexigenic effects is agouti-related protein (AgRP). This neurotransmitter has been shown to have a potent effect on food intake [6] and is also thought to be important in control of energy homeostasis [7] stress effects, thermoregulation, pain, and reproduction [6].

Historically, the hypothalamus has been significantly implicated in the control of food intake. The PVN has been shown to be an especially potent site for pharmacological stimulation of feeding behavior, and receives innervation from fibers containing several neurotransmitters involved in food intake. NE projections originating
in the hindbrain [8] have been shown by neuroanatomical studies to innervate the PVN [9]. Additionally, the PVN has been shown to contain one of the densest populations of NPY terminals in the brain [10]. Afferent NPY fibers from brainstem nuclei (C1, A1, C2, and C3) and the arcuate nucleus of the hypothalamus project to the PVN [4]. AgRP is synthesized only in the arcuate nucleus [5], and AgRP cell bodies always co-express NPY [5, 6]. These neurons densely innervate the PVN, such that approximately 50% of the NPY released in the PVN is released by arcuate NPY/AgRP neurons [5]. Additionally, AgRP is most effective at stimulating food intake when injected into the PVN, suggesting that the PVN may be one of the major sites of action of AgRP in controlling feeding behavior [11]. Overall, the PVN is a prime site for investigation into the role of NE, NPY, and AgRP in consummatory feeding behavior.

The neurotransmitters NE, NPY, and AgRP have all been shown to have a potent orexigenic effect on feeding behavior, especially when pharmacologically injected into the site of the PVN. Past research has attempted to separate appetitive and consummatory feeding behavior by using an intraoral subcutaneous cannula [12]. Findings have suggested that while the neurotransmitters NE and NPY increase appetitive food intake, they decrease consummatory intake [13, 14, 15, 16].

The notion of opposing regulation of consummatory and appetitive feeding behavior seems counterintuitive, and further investigation of the effects of NE and NPY on consummatory feeding behavior are necessary for understanding of the neural circuitry of feeding behavior. Additionally, the effects of AgRP on consummatory feeding behavior have not been investigated. Establishing AgRP's role is also necessary for understanding the mechanisms and pathways of feeding behavior, considering that
NPY and AgRP are co-localized in the PVN. Woods et al. speculated that a decrease in consummatory behavior may have been the result of an aversive effect to large doses of NPY [15]. To counteract this potential effect, this study utilizes a significantly smaller dose of NPY that, while smaller, has still been shown to have an orexigenic effect on appetitive feeding behavior [10]. In addition, the peptides in this study were injected into the PVN, instead of intracerebroventricular (ICV). Injections into the PVN serve to minimize non-specific effects caused by diffusion of peptides injected ICV.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats from Harlan Laboratories weighing 280-320 g at the start of the experiment were used. The rats were housed in individual suspended wire mesh cages under standard AAALAC-approved conditions in a temperature-controlled room kept at 21°C. The room was lit on a 12:12 light-dark cycle. Rats were permitted ad libitum access to pellet rat chow (Harlan Teklad F6 Rodent Diet W, Madison, WI) and water, except during testing. Tests were conducted between 1400 and 1700 h. All experimental animal protocols were approved by the Washington State University Institutional Animal Care and Use Committee, which conforms to National Institute of Health rules and regulations.
**Preparation of animals**

The rats were anesthetized with a ketamine cocktail consisting of 5 mL ketamine, 2.5 mL rompun, 1 mL acepromazine, and 1.5 mL saline that was injected 0.1 mL per 100 g rat body weight. They were then fitted with a cheek fistula designed and constructed at Washington State University (Fig. 1) in their right cheek just lateral and rostral to the first premolar using a 16-gauge needle to pierce the tissue [17]. After one week of recovery, the rats were habituated to intraoral feeding through the cheek fistula. The rats were anesthetized for intracranial cannula placement using the ketamine cocktail. Cannulas were 15 mm in length and made of 26-gauge stainless steel tubing. Cannulas were implanted into the right PVN (coordinates 7.4 mm ventral to dura mater, 1.8 mm caudal to bregma, ±0.4 mm lateral from midline) using a stereotaxic instrument that assisted in locating specific skull landmarks, such as the bregma line. A scalp incision was made, and a small localized hole was drilled through the skull through which the cannula was lowered into the neural tissue. Three small holes were then drilled into the surrounding skull, and small screws were secured into the holes. Dental Acrylic cement was applied to the crown of the skull to hold the cannula in place. While not in use, a 33-gauge plastic obturator was kept in the cannula. Rats were allowed one week of recovery before experimentation.
Figure 1. Drawings depicting the design and implementation of the stainless steel cheek fistula used for intraoral delivery of liquid food. The components of the fistula are shown in diagrams A, B and C. The fistula itself (A) was constructed from a stainless steel rod (0.07" diameter, 0.33" long), drilled through the center (#57 drill) to create a 0.043" diameter lumen, and threaded (#2-56 threading) along its exterior surface. A washer (0.02" thick) was permanently affixed to one end (the intraoral end) of the rod. The infusion tip (B) contained a central lumen compatible in diameter with the fistula. It was threaded (#2-56) at one end so that it could be screwed onto the externalized portion of the fistula during infusion of liquid food. The opposite end of the infusion tip was beveled (0.09" largest diameter) to facilitate the attachment of silastic tubing used to deliver the food. A threaded washer (C) was used to hold the fistula in place after removal of the infusion tip. The washer was notched to facilitate manipulation of the washer with forceps. Diagram D shows the fistula assembled for infusion, with the infusion tip attached. Diagram E shows the fistula assembly between experiments, with the infusion tip removed and replaced with the washer.

Injection procedure

For intracranial delivery of solutions containing the neurochemicals of interest or control solution an injection cannula 0.5 mm longer than the guide cannulas was made from 33-gauge stainless steel tubing. At the time of injection, injectors were attached to
10 μL Hamilton microsyringes via 30.5 gauge polyethylene tubing and inserted into the guide cannula. Injection volume was 200 nL for all injections. Injectors were left in place for an additional 60 seconds after delivery of neurochemicals to ensure diffusion from the cannula tip. Rats were returned to their home cages until feeding tests commenced, as described below.

**Experiment 1: Intraoral feeding in response to NPY injection into PVN**

Food was removed 60 minutes prior to testing. To measure baseline consumption, rats were injected intracranially with 200 nL 0.9% saline control solution and returned to their home cages. Eight minutes following injection the intraoral fistulas were fastened to 60 mL syringe pumps via polyethylene tubing, and behavior testing was initiated. Syringe pumps were set to deliver liquid food (40% lactose free whole milk, diluted with tap water) at a rate of 1mL/minute. Intraoral infusion was interrupted every 5 minutes during the test. Rats were judged to be satiated when the milk solution dripped from their mouths. After one drip, testing was paused for 1 minute. If the rat rejected the milk solution from his mouth within 30 seconds following the 1 minute break, testing was terminated, and the final baseline volume was recorded. Two days following the baseline injection, rats were injected with 78 pmol NPY (200 nL) and tested for intraoral intake as described above.

**Experiment 2: Intraoral feeding in response to NE injection into PVH**

Food was removed 60 minutes prior to testing. Rats were injected with 40 nmol NE (200 nL), and intraoral intake was measured as described in Experiment 2.
Experiment 3: Intraoral feeding in response to AgRP injection into PVH

Two hours prior to testing food was removed and 200 pmol AgRP (200 nL) was injected intracranially. The injection schedule was based on studies showing that the effects of AgRP peak after a 2-hour delay following injection into the hypothalamus [18]. Intraoral intake was measured as described in Experiment 2.

Verification of cannula placement

Rats were sacrificed by administration of a lethal dose of halothane anesthesia. A perfusion procedure following the conclusion of the experiments. At the point of death (indicated by the cessation of respiration) an abdominal incision was made allowing the rib cage to be pulled away exposing the diaphragm. An incision through the diaphragm was made to expose the heart. A 16-gauge needle was placed into the left ventricle and a small incision was made in the right ventricle allowing the blood to leave the body. The body was then infused with a 0.1 M phosphate buffer solution consisting of double distilled water, sodium chloride, and 0.4 M phosphate buffer. Once the circulatory system was flushed, a formaldehyde fixative solution consisting of double distilled water, 0.4 M phosphate buffer, and 37% formaldehyde was then infused through the system. Both solutions were made to a pH of 7.4. After the tissue was thoroughly fixed, the brain was extracted from the skull, placed into 15ml of tissue fixing solution, and stored in a refrigerator until ready for sectioning.

Twenty-four hours prior to sectioning, the brains were placed in a 25% sucrose, phosphate buffer, and double distilled water solution overnight. The brains were frozen
onto a slide using 0.25% gelatin. The brains were cut using a microtome (Histostat 855) to a thickness of 40um and directly mounted onto microscope slides. After drying, the sections stained with cresyl violet and coverslipped following the procedure outlined by Paxinos and Watson [19]. The slides were immersed for five minutes each in xylene, xylene again, 100% ethanol, 95% ethanol, and 70% ethanol. They were then dipped in double distilled water (ddH₂O) and stained with a Cresyl violet solution for 15 minutes. The slides were then placed into each of the following for approximately five minutes each: ddH₂O, 70% ethanol, 95% ethanol, 100% ethanol, xylene, and xylene again. The slides were coverslipped using DPX Mountant. Cannula placements were verified via the Paxinos and Watson 4th edition Rat Brain Atlas [19].

Diffusion Radius of Injected Solutions

Two additional male Sprague-Dawley rats were implanted with PVN cannulas as described above. The rats were allowed five days to recover and then were injected intracranially with Fluoro-Gold using the injection procedure described above. Fluoro-Gold, a fluorescent marker, was used to estimate the diffusion radius of the solutions (200 nL) injected into the hypothalamus. Two hours following intracranial injection, the rats were perfused and the brains were sectioned in the manner described above. The brains were sectioned as described above; however, the sections were stored in TPBS rather than directly mounted onto slides for immunohistochemical analysis.

Immunohistochemistry
The sections were removed from the TPBS solution and placed into 70% ethanol for 30 minutes. The sections were then rinsed three times with the TPBS solution, allowing them to sit in TPBS for approximately 5 minutes per rinse, and placed into 1mL of 10% normal horse serum (NHS) for 30 minutes. The sections were then placed into a solution containing the primary antibody, Fluorogold α rabbit, and 10% NHS in a concentration of 1:50K and were left for 72 hours to incubate in the solution. The sections were rinsed three times with TPBS in the same manner described previously and placed into a solution containing the secondary antibody, biotinylated donkey α rabbit, and 1% NHS with a concentration of 1:500. After incubating for 24 hours in the above solution, the sections were rinsed three times in TPBS, and placed in ExtrAvidin®-Cy3 Conjugate 1:1500 TPBS solution for 24 hours. The sections were then rinsed three times in TPBS, and 2 mL of 3,3 diaminobenzidine tetrahydrochloride (DAB) were placed in each vial to incubate for 10 minutes. Two microliters of glucose oxidase were added to each vial. The reaction was stopped with TPBS when the sections showed an intermediate-colored black stain. After rinsing three times with TPBS, the sections were mounted onto slides.

The sections were viewed using a fluorescence microscope fixed with an ultraviolet excitation filter at 323 nm. Fluoro-Gold emits at 408 nm. Using Paxinos and Watson 4th edition Rat Brain Atlas and the fluorescence microscope, the radius of Fluoro-Gold diffusion was found to be 1.255 mm rostral-caudally, and 0.588 mm laterally. All animals whose cannula placements did not fall into the radius of diffusion to the PVN indicating that the neurochemicals would not have reached their intended targets, were
not used in the data analysis. This procedure provided a mechanism for eliminating false negative cases from the data set.

Statistics

Feeding responses were analyzed by the NCSS statistics software program. A one-way repeated measures ANOVA test was used followed by the Bonferroni pairwise multiple comparisons procedure. Differences were considered significant if $P < 0.05$.

Results

Rats recovered rapidly from both intraoral fistula and intracranial cannula surgeries. On the day following surgery rats were fully mobile and feeding normally. Injections and behavior testing began one week following surgery. All rats used for behavior testing were healthy during the course of testing, continued to show normal weight gain, and did not exhibit any neurological impairment.

All intracranial cannulas were located in or around the PVN, as determined by cresyl violet staining and microscopy (Fig. 2). In addition, injections of Fluoro-Gold were done to determine whether injected substances would sufficiently disperse within the PVN. Fluoro-Gold experiments verified the dispersion radius of injected substances within hypothalamic tissue to be 1.255 mm in the rostral-caudal axis and 0.588 mm in the lateral axis. All cannula placements fell within this dispersion radius from the PVN, therefore all substances injected in our experiments adequately dispersed to the PVN.
Figure 2. Verification of Cannula Placements. All cannula sites were mapped using the Paxinos and Watson 4th edition rat brain atlas and were found to be located as shown.
Intracranial injections of NE (40 nmol/200 nL) significantly increased intraoral intake as compared to 0.9% saline (P<0.05). Controls consumed 13.2 ± 2.64 mL of milk in response to saline and 27.5 ± 4.98 mL following NE injections (Fig. 3). In addition, injection of NPY (78 pmol/200 nL) also significantly increased intraoral intake (P<0.05). Rats consumed 24.1 ± 4.61 mL of milk following NPY injections (Fig. 4). Finally, injections of AgRP (200 pmol/200 nL) into the PVN significantly increased intraoral intake (P<0.05). Rats drank 30.7 ± 6.04 mL following AgRP injections (Fig. 5).

Figure 3. Cumulative intraoral intake of 40% lactose free milk solution infused through an intraoral fistula beginning eight minutes following intracranial injection of 0.9% Saline (200nL) and norepinephrine (NE) (40 nmol/200 nL) respectively. Rats significantly increased their intake of milk in response to NE. Data are expressed as mean intraoral intake in mLs ± SEM. (p < 0.05 vs intake after saline).
Figure 4. Cumulative intraoral intake of 40% lactose free milk solution infused through an intraoral fistula beginning eight minutes following intracranial injection of 0.9% Saline (200nL) and neuropeptide Y (NPY) (78 pmol/200 nL) respectively. Rats significantly increased their intake of milk in response to NPY. Data are expressed as mean intraoral intake in mLs ± SEM. (p < 0.05 vs intake after saline).

Figure 5. Cumulative intraoral intake of 40% lactose free milk solution infused through an intraoral fistula beginning eight minutes following intracranial injection of 0.9% Saline (200nL) and two hours following intracranial injection of Agouti-related Protein (AgRP) (200pmol/200 nL) respectively. Rats significantly increased their intake of milk in response to NPY. Data are expressed as mean intraoral intake in mLs ± SEM. (p < 0.05 vs intake after saline).
Discussion

In this study, the potent orexigenic neurotransmitters NE, NPY, and AgRP were found to increase the consummatory feeding response when injected into the PVN, suggesting that there exists an efferent projection from the PVN to consummatory feeding centers located in the hindbrain.

These results are in direct opposition to previous studies which have found that NPY decreases consummatory feeding [13, 14, 15, 16]. A lower dose of NPY injected directly in to PVN rather than ICV effectively eliminated non-specific effects caused by diffusion of NPY ICV, thereby increasing the validity of our data. In addition, we altered the diet used in testing intraoral intake. We used a dilute milk solution that proved to be highly palatable and removed the limitation on intake caused by negative osmotic effects associated with hypo and hypertonic solutions. Data planned for publication from our lab has shown 15% sucrose solutions elicit maximum intake. Therefore we can conclude that 3% [13] and 30% [14] sucrose solutions are less than optimal for testing intraoral intake.

Data from this study supports the idea that NE, NPY and AgRP- containing pathways are likely mediators of the adiposity signals controlling the complex energy homeostasis circuit [20]. Energy homeostasis in the body is in part maintained by strict food intake regulation. It is now speculated that there are two major adiposity signals in the body, insulin and leptin, which work to signal to the brain the amount of energy available stored in the form of fat. Leptin is secreted from adipose tissue and regulates body mass by acting directly on neurons of the arcuate nucleus of the hypothalamus. Leptin has an inhibitory effect on NPY and AgRP hypothalamic neurons, and decreased leptin expression increases NPY and AgRP activity. This finding demonstrates that the
effects of NPY and AgRP on food intake are important components of the energy homeostasis system [20]. Previous research has verified the stimulatory role of NE, NPY, and AgRP in the appetitive component of feeding behavior and energy homeostasis. Data from this study specifically suggests that NE, NPY, and AgRP have a similar stimulatory role in the consummatory component of feeding behavior and energy homeostasis.

Previous work has shown that insulin-induced hypoglycemia elicits consummatory feeding in decerebrate rats (in which the forebrain is disconnected from the midbrain and hindbrain). These results indicate that higher order forebrain mechanisms are not essential for the production of consummatory feeding behaviors in response to some ingestive signals and demonstrate that motor circuitry controlling consummatory responses is located in the caudal brainstem [21]. The neural circuitry controlling reflexes associated with the consummatory response has been shown to be located in the following hindbrain areas: nucleus of the solitary tract (NTS), nucleus ambiguous (NA), and reticular formation (RF) of the caudal pons and rostral medulla [22]. Also located in the hindbrain and adjacent to these swallowing loci are nuclei controlling other basic, unlearned bodily functions such as respiration and heart rate. Studies using weaning-age rats have indicated that like these basic functions, consummatory feeding behavior is inherent and present at birth, whereas appetitive feeding behavior is learned [23]. Thus it appears that centers controlling consummatory feeding are located in lower order brain areas, like the hindbrain, while appetitive feeding behavior is most likely controlled by higher order neural areas, including the hypothalamus and basal forebrain.
While the existence of afferent adrenergic and NPY projections from the hindbrain to the PVN has been established [9, 4], data from this study proposes either the existence of efferent NPY and AgRP projections from the PVN to the hindbrain consummatory control centers or the existence of AgRP, NE, and NPY terminals in the hypothalamus that may innervate neurons descending to hindbrain consummatory reflex control sites. The existence of efferent projections suggests that consummatory feeding centers may receive regulatory input from higher order forebrain areas like the PVN that most likely serves to integrate appetitive and consummatory feeding. Studies also have shown that NPY receptors are located in swallowing control areas, such as the NTS [24]. In addition, Bagnol, et al [6] found through immunohistochemistry results that AgRP fibers and terminals are present in light density in the NTS and moderate density in the NA, two hindbrain areas previously discussed to be sites of consummatory reflex control [22] and the sensation of blood glucose levels [25]. These findings, coupled with the results of this study, suggest the existence of efferent NPY and AgRP projections from PVN to hindbrain control sites that increase consummatory feeding responses.

Further research on this topic is needed. Our effects were found to be significant using a strict statistical analysis (the Bonferroni pairwise multiple comparisons procedure), despite the relatively small number of subjects. This indicates a highly robust effect of the neurochemicals on food consumption. Nevertheless, repeating this study with more animals would strengthen the findings by allowing us to map both positive and negative effects and thus to localized effective injection sites to specific anatomical structures and to relate those functional maps to maps of NE, NPY, and AgRP terminal distribution within the hypothalamus. Another limitation was the stereotaxic
approach to cannula implantation which is subject to variability due to anatomical variation in the rat skull and brain features. The next step in this research would be to inject NE, NPY, and AgRP into hindbrain sites implicated in the consummatory feeding component and swallowing reflex, such as the NTS or NA, or lesioning of these loci and test the effects on consummatory behavior. This would allow us to directly test the hypothesis that the signal originating from the hypothalamus injection site is transmitted to the hindbrain for control of sites involved in consummatory reflexes. Such a study would strengthen results from this research.

**Conclusion**

In summary, injections of the hypothalamic peptides norepinephrine, Neuropeptide Y, and agouti-related protein into the paraventricular nucleus of the hypothalamus elicit a significant increase in consummatory feeding behavior. This finding suggests pathways descending from the forebrain area of the PVN to hindbrain sites controlling the mechanics of consummatory feeding. This pathway is probably responsible for integration of forebrain appetitive behaviors and hindbrain consummatory behaviors, and is an important component in mapping the circuitry of the neural control of food intake. Greater understanding of this circuitry will lead to advances in the treatment of disorders related to energy homeostasis, such as obesity and diabetes.
References


food intake: Discrepancies in the model. Regulatory Peptides 1998; 75-76: 403-408.


REQUEST FOR PERMISSION TO INCLUDE YOUR HONORS STUDENT’S THESIS IN THE WSU RESEARCH EXCHANGE

Washington State University Libraries

Permission is requested for a non-exclusive license to post the Honors thesis described below in digital form in the Honors College community within the WSU Research Exchange <https://research.wsulibs.wsu.edu:8443/dspace/community>. Posting in the Research Exchange will make the material publicly available as part of the Washington State University Research Exchange digital repository of research-related documents. Additional information about Research Exchange can be viewed at <http://research.wsulibs.wsu.edu>.

Thesis Author ___________ Carter, Kelli ___________

Thesis Title The Role of Hypothalamic Peptides in Consummatory Feeding Behavior

Date of Thesis ___________ Spring 2004 ___________

I grant a non-exclusive right to include this item in the Research Exchange. All other rights under copyright law are retained.

Permission granted by:

Sue Ritter ___________

Name (please print) ___________

355-8113 ___________

Telephone / Email (sitter@wsu.edu)

Signature (not computer generated) ___________

Date of Signature ___________

Please return the form with your written signature via fax, email, or postal service to:

Kay Vyhnaneek, Scholarly Communication Librarian
120F Terrell Library / PO Box 645610
Washington State University
Pullman, WA 99164-5610 USA
Fax: +1-509-335-672