KAPPA AND DELTA OPIOID RECEPTORS IN THE LATERAL SEPTUM IN THE CONTROL OF FEEDING

By

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Abstract

According to 2017 data from the National Health and Nutrition Examination Survey, the prevalence of obesity increased to 39.8% in adults and 18.5% in youth in 2015-2016. The major health risks of obesity include diabetes, heart diseases, stroke, and increased cancer risk. Possible dysregulation of neural circuits can give rise to not only excessive eating but dangerous eating disorders such as anorexia nervosa and bulimia. This prompts the importance of researching underlying mechanisms responsible for feeding or disordered feeding. One region of the brain called the lateral septum (LS) has the potential to play an integrative role in several brain regions related to feeding, the hypothalamus in particular. Previous research shows an increase in food intake in response to stimulation of mu opioid receptors in the LS, suggesting an important role for opioid receptors of the LS in feeding. Kappa and delta opioid receptors are also found in the LS and could potentially play a role in the septal control of feeding. Injecting kappa and delta opioid receptor agonists into the lateral septum with a wide range of doses did not have a significant effect on feeding in rats. This would support the idea that mu opioid receptors are the main component involved in morphine elicited feeding.

Acknowledgments

I would like to thank Dr. Glenn Stanley for allowing me to be a Research Assistant in his lab for the past three years. I appreciate all the motivation and support throughout my time as an undergraduate student at UCR. I also want to thank my fellow undergraduates Roshini Patel, Qi Chu, Victoria Cha, and Rohit Jonnalagadda. Special thanks to Michelle Calderwood for allowing me to work closely alongside her research. This project would have been impossible without her invaluable guidance and her canine stress relief. Finally, special thanks to Andy Tseng for accepting me into this lab and teaching me the advanced techniques to succeed. Although I'll be leaving this lab with an acquired allergy to rats, I wouldn't hesitate to do it all over again. Thank you all for the memories.

Table of Contents

Introduction:

Obesity is a growing health concern in the United States. According to 2017 data from the National Health and Nutrition Examination Survey, the prevalence of obesity increased to 39.8% in adults and 18.5% in youth in 2015-2016. The major health risks of obesity include diabetes, hyperlipidemia, hypertension, chronic heart diseases, stroke, and increased risk of some forms of cancer. The mechanisms underlying feeding motivation continue to be poorly understood. This includes the familiar urge to continue eating even after a full meal or the inclination to eat due to emotional distress. This research may help with efforts to reduce the disillusioning growth of obesity in America. My project ultimately aims to explore the neural mechanisms underlying disordered and unhealthy eating.

More specifically, my research will focus on a brain region called the lateral septum (LS) which may have a large influence on motivational behavior. A prior study for the LS implication in neuropsychiatry supports that improper functioning of the LS affects motivation in rats (T.P Sheehan et al, 2004). The LS has extensive neural connections with regions of the hypothalamus that play major roles in regulating appetite and hunger. The LS is predominantly composed of GABAergic projection neurons that interconnect with the lateral hypothalamus, a key region particularly sensitive to reward and appetitively motivated behaviors (Numan, 2012). Recent work has also shown that optogenetic stimulation of LS projections to the lateral hypothalamus stimulates eating (Sweeney et al, 2016). Therefore, the LS appears to have a fundamental impact on feeding behavior.

Opiates are known to influence dopamine levels in the brain by inhibiting GABA neurons. GABA neurons inhibit the dopaminergic neurons that densely occupy the ventral

tegmental area, an important structure of the reward pathway. Therefore, as opiates inhibit GABA neurons, it allows for dopaminergic neurons to fire more frequently. Several types of experimental obesity are associated with increased opiate production and/or increased sensitivity of opiate receptors. This is largely attributed to how the effect of opiate stimulation could mirror the rewarding sensation of eating. Specific palatable macronutrients such as sugar has been known to have significant effect on feeding behavior when mediated by opiates.

Thus, opioid receptors in the LS could play a role in the integration of contextual, emotional, and rewarding information from multiple brain areas to modulate feeding. Previous research in Dr. Glenn Stanley's lab has shown that activation of mu-opioid receptors in the LS by intracranial morphine injection increased eating behavior in rats (Stanley et al, 1988). Two separate types of opioid receptor, kappa and delta also densely occupy the LS (Risold et al, 1997) suggesting that they may modulate feeding behavior.

The purpose of my study is to better understand how the LS may be modulating feeding behavior. More specifically, I will determine whether stimulation of LS kappa and delta opioid receptors, like the stimulation of mu opioid receptors, will elicit eating behavior. Although morphine has a high affinity for mu-opioid receptors, it also binds kappa- and delta-opioid receptors with less affinity. Opioid receptor agonist increases eating in several brain regions that mediate food intake control (Glass et al, 1999). A preliminary study in our lab also found a significant increase in feeding is produced by LS injection of a highly specific mu-opioid receptor agonist, with no significant increases produced by low doses of either a highly specific delta-opioid agonist or a kappa-opioid agonist. My study was designed to further investigate LS delta- and kappa-opioid receptors in feeding stimulation by using a wider range of doses of the

highly selective kappa- and delta- agonists. We used male Sprague-Dawley rats to measure the effect on feeding with LS injections of each agonist. I hypothesized that neither kappa- nor delta-opioid receptor agonists would elicit feeding. If correct, that would provide support for the hypothesis that mu-opioid receptors mediate the eating elicited by morphine injected into the LS.

Methodology:

Animals

20 adult male Sprague-Dawley rats were used for this experiment. Rats were bred in an on-campus vivarium on a 12-hour light/12-hour dark cycle. They were maintained on Purina rat chow until surgery. After a week of acclimating to the single housed cages, the animals were anesthetized with intraperitoneal sodium pentobarbital and atropine in preparation for stereotaxic surgery. The animal was secured into the machine with ear bars. Then, the animal's scalp was cut open with a surgical scalpel and the animal was further anesthetized with topical lidocaine gel. A stainless-steel guide cannula was placed 1.0 mm dorsal to the LS target site and held in place with skull screws and dental acrylic. The stereotaxic coordinates for the LS are: 0.3 mm anterior to bregma, 0.8 mm lateral to the midline, and 4.8 mm ventral to the surface of the skull. Animals were then given one week to recover from surgery. They were handled and given mock injections prior to testing to help them acclimate to the injection experiments.

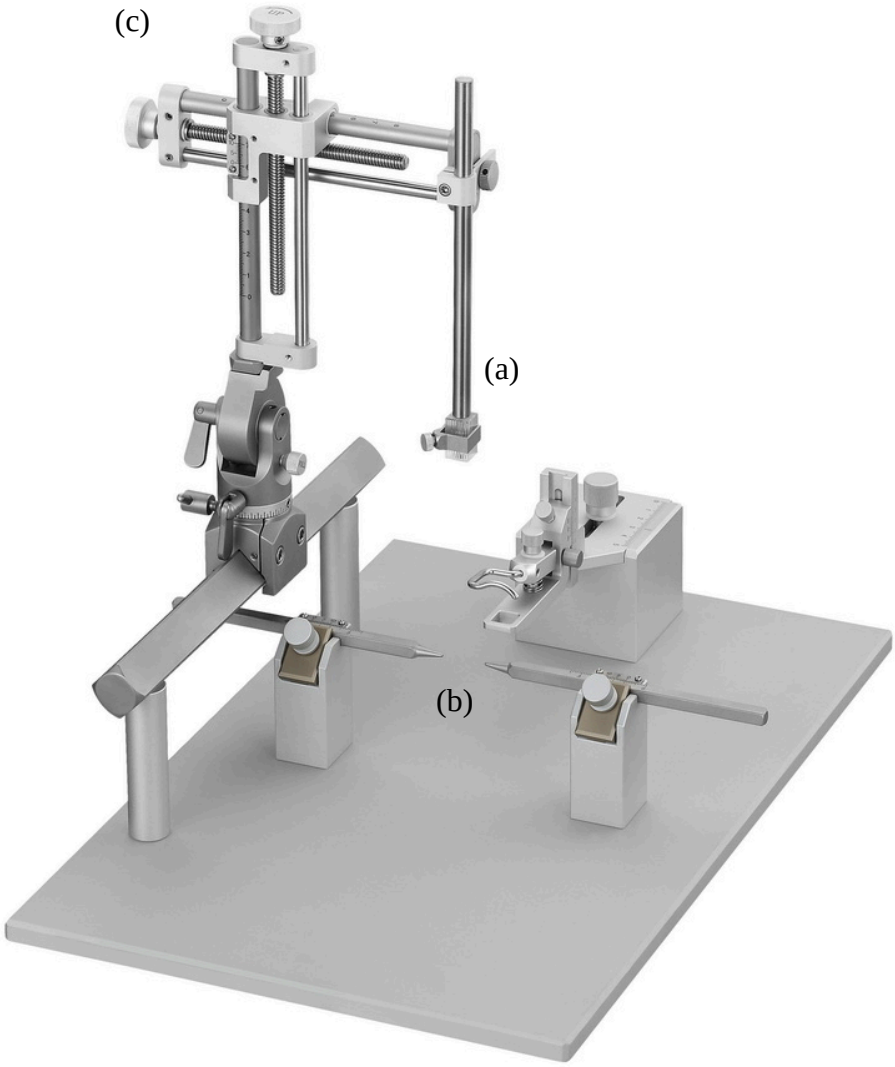

Figure 1. Stereotaxic Instrument used to perform surgery. This is a technique to accurately target deep structures within the brain using 3D coordinates with respect to anatomical landmarks on the skull. (a) Arm for cannula placement. (b) Ear bars for fixation of the rat to the machine. (c) Knobs for adjustment of stereotaxic coordinates

Procedure

Experiment 1: DPDPE (delta-opioid agonist) injections

Dose 1: 0.125 µg/µL

Dose 2: 0.5 µg/µL

Dose 3: 0.75 µg/µL

Dose 4: 1.0 µg/µL

Experiment 2: U50488H (kappa-opioid agonist) injections

Dose 1: 0.075 µg/µL

Dose 2: 0.3 µg/µL

Dose 3: 0.6 µg/µL

Dose 4: 1.0 µg/µL

Three days prior to testing, animals were given a mash diet consisting of ground Purina rat chow, evaporated milk, and sugar. Animals were tested under satiated conditions and given fresh mash diet one hour prior to injections. Experiment 1 tested delta-opioid agonist DPDPE on 10 Sprague-Dawley rats, by injecting aCSF, Dose 1, Dose 2, Dose 3, and Dose 4 on 5 separate days. Similarly, Experiment 2 tested 5 doses over separate days of kappa-opioid agonist U50488H (See above for dosage amount). Agonist drug was injected through the cannula using a stainless steel microinjector connecting to a glass syringe via polyethylene tubing. Each drug was injected through the cannula and onto the LS target site with injectors that extend 1.0 mm beyond the guide cannula. All the subjects were tested with each dose of DPDPE or U50488H once in counterbalanced order. Measurements of food consumption were taken every 0, 30, 60, 120, and 180 minutes post injection of agonist and control.

Evaluation

Location of cannula placement was verified by extraction and staining of the animal's brain following testing. Animals were injected with 0.6 mL of FATAL-PLUS solution for euthanasia. Immediately after being anesthetized, the animals were transcardially perfused with formalin into the left ventricle to preserve and fixate the brain tissue. After perfusing for about 15 minutes, the brain was extracted from the skull and stored in formaldehyde. Brains were then frozen with dry ice and sectioned with a microtome into 100 µm coronal slices. Slices were immediately submerged into gel and mounted onto slides to be dried overnight. Slides were then stained with Cresyl-violet for visualization and verification of cannula placement. Measurements of food intake were compared for all 20 animals in the study. Four Two-way ANOVAs were run to compare groups across treatment days at each time point.

Results:

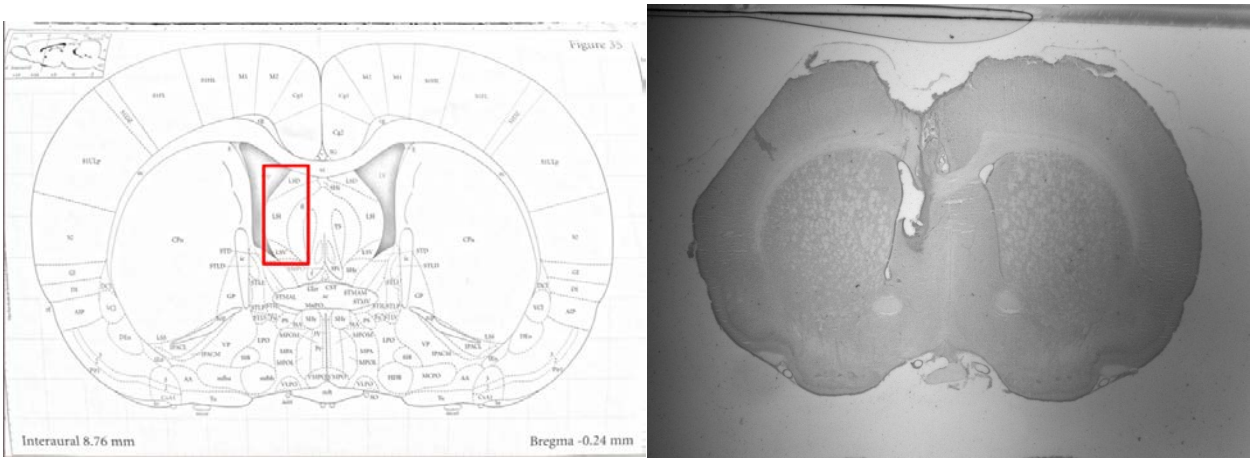

Figure 2. This is a coronal section diagram obtained from Paxinos' and Watson's Rat Brain Atlas outlining the location of the lateral septum (LEFT).

This is a 100µm section of a rat brain with the successful placement of the stainless-steel cannula 1 mm above the lateral septum (RIGHT).

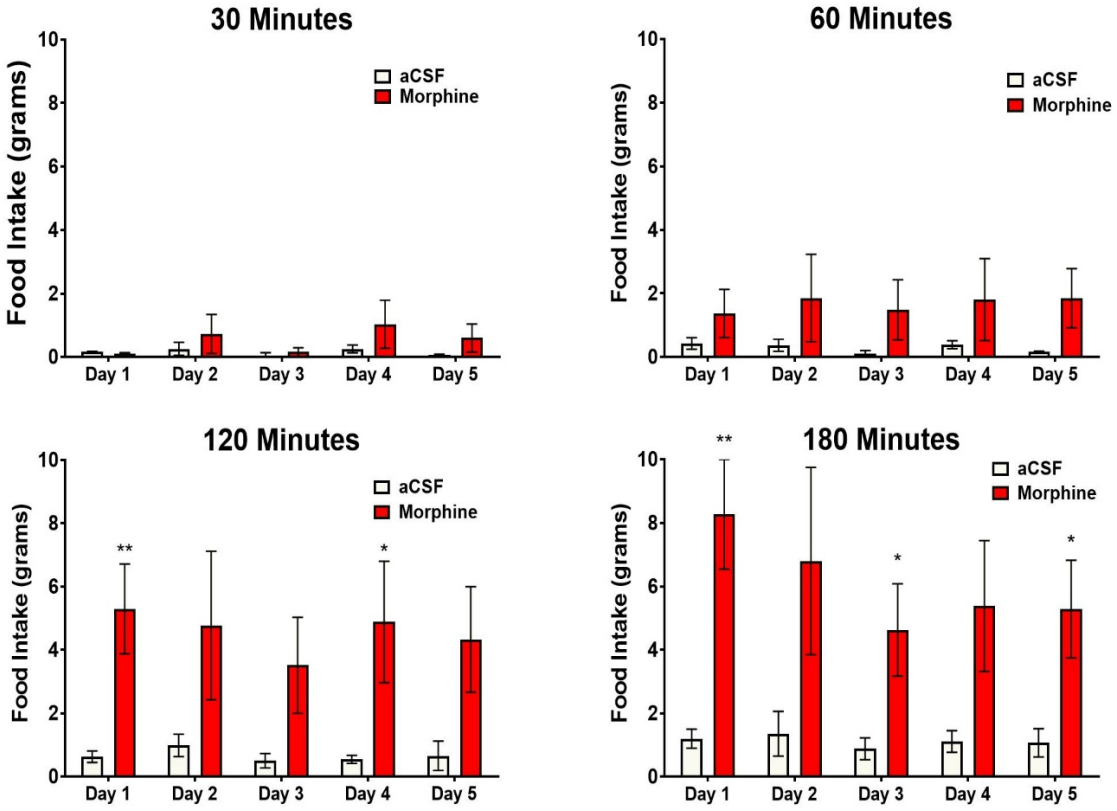

Figure 3. This previously conducted study tested the animals' feeding response to intracranial injections of morphine vs aCSF in the LS. At 30- and 60-minutes post-injection there were no significant changes in food intake for animals receiving aCSF or morphine. However, there was a significant difference in food intake across all 5 testing days with animals receiving morphine compared to those receiving aCSF at 120- and 180-minutes post-injection.

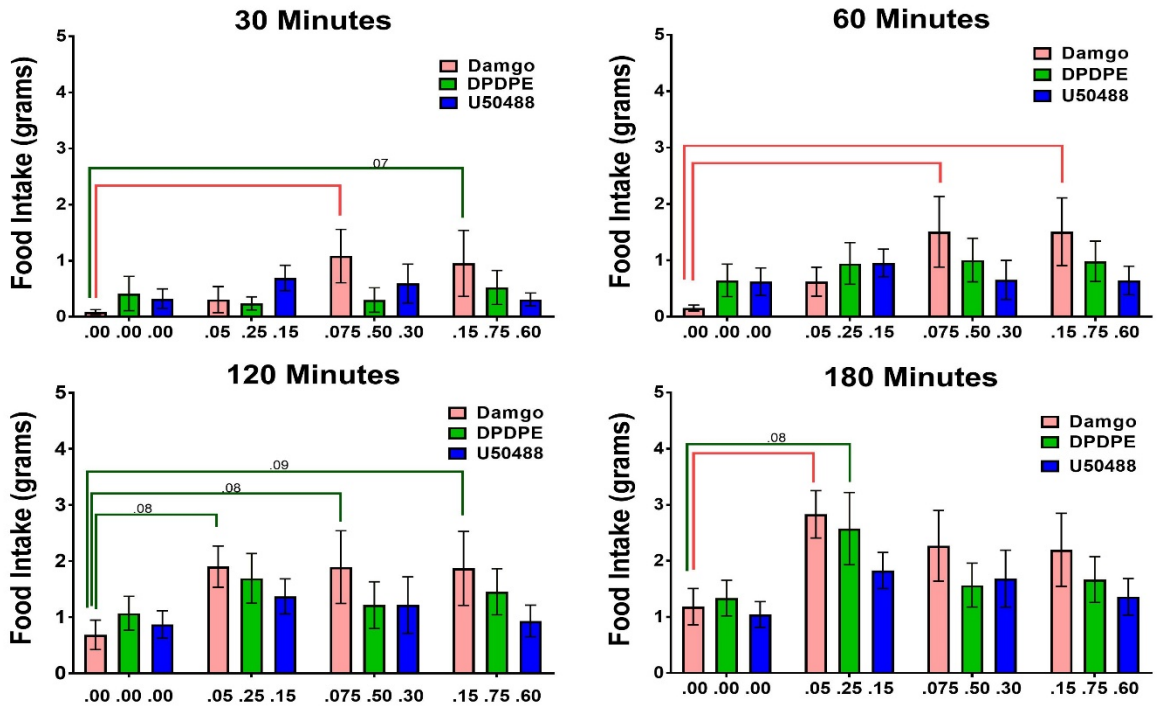

Figure 4. This preliminary study tested 3 doses of the opioid receptor agonists for mu (DAMGO), delta (DPDPE), and kappa (U50488H) receptors. At 30- and 60-minutes post-injection, animals receiving DAMGO showed a significant increase in feeding compared to those receiving DPDPE, U50488, and especially aCSF. At 120 minutes post-injection, DAMGO animals continued to show trending food intake over all 3 dose ranges. At 180 minutes, DAMGO at its lowest dose significantly increased eating. Interestingly, DPDPE showed a trend towards increased food intake for the 0.25µg/µL dose when compared at 180 minutes post-injection ($p=0.08$).

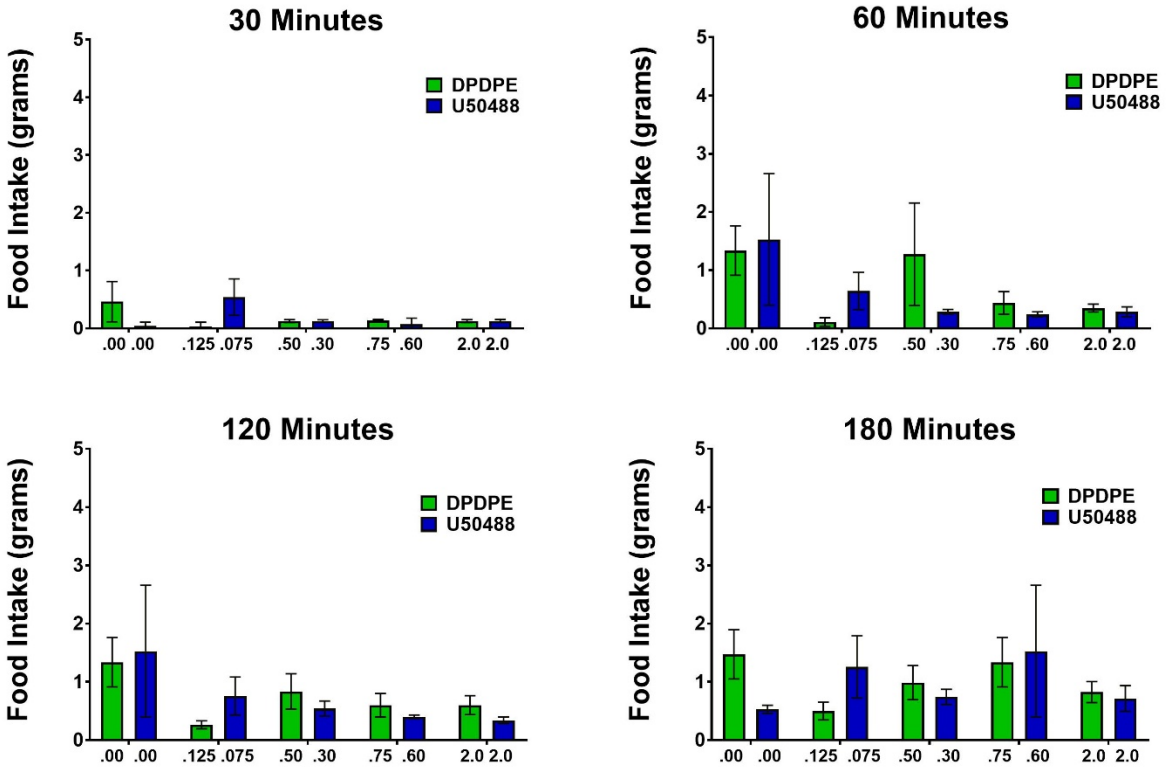

Figure 5. This data represents my subsequent study of delta and kappa agonists over broader dose ranges conducted over 5 testing days. No dose of either DPDPE or U50488 produced a significant increase in food intake compared to the aCSF control (.00 µg/µL).

Figure 3 was a prior study of rat's food intake after morphine injection compared to the food intake following aCSF injection. Food intake was measured every 30-, 60-, 120-, and 180-minutes for each of the 5 days of injections. At 30 and 60 minutes, there was no significant increase in food intake. However, there is a noticeably increased feeding trend in morphine injected rats at the 60-minute mark. After 2 hours, there was a much more apparent change in feeding with morphine injected rats. At 120 minutes, there was a significant increase in eating with the morphine group compared to the aCSF control group particularly on Days 1 and 4.

There continued to be a significant increase in food intake for Day 1 at 180 minutes as well as rats on Day 3 and 5.

Figure 4 was another preliminary study conducted in a similar manner as the morphine study but measuring food intake after injection with specific opioid agonists DAMGO, DPDPE, and U50488H for mu, delta, and kappa opioid receptors, respectively. The rats were injected with aCSF as the control group and 3 varying doses of each of the agonists. At 30 minutes, DAMGO increased feeding in with a dose of 0.075 µg/µL and a trending increase in 0.15 µg/µL ($p=0.7$). Significant increase in food intake continued for these doses when food was measured at 60 minutes. At 120 minutes, all three DAMGO doses at 0.05 µg/µL, 0.075 µg/µL, and 0.15 µg/µL have a trending increase in feeding ($p=0.8$, $p=0.8$, $p=0.9$, respectively). DAMGO shows a significant increase in feeding in the final measurement at 180 minutes as well for the lowest dose of 0.05 µg/µL. U50488H showed no change in feeding across all doses of testing compared to the control. DPDPE had no change in feeding until the last measurement at 180 minutes that showed a trending increase in food intake at 0.25 µg/µL ($p=0.08$).

Figure 4 shows the food intake of rats given injections of delta and kappa opioid agonists, DPDPE and U50488H. This was conducted over 5 days using aCSF as control and 4 varying doses of agonist. Measurement of food intake taken at 30-, 60-, 120-, and 180-minute intervals indicated no apparent increase in feeding compared to the control group for both DPDPE and U50488H.

Discussion:

Data gathered from the preliminary studies in Figure 3 strongly suggests that morphine has a significant increase in food intake when injected into the LS compared to aCSF. The

largest increase in food intake was most revealing after 120- and 180-minute measurements. Animals were then tested comparing various doses of DAMGO, DPDPE, and U50488H, the opioid agonists for mu, delta, and kappa receptors, respectively as shown in Figure 4. An increase in feeding was seen consistently for DAMGO during food intake measurements at 30-, 60-, 120- and 180-minutes. Since DPDPE had a trending increase in food intake at the 120-minute measurement, it was proposed to take an additional experiment comparing the stimulation of only delta and kappa receptors at adjusted concentrations. Thus, my experiment was conducted to assess the response to DPDPE and U50488H specifically at broader dose ranges. Figure 5 details that there was no significant increase in eating for DPDPE nor U50488H injected rats. This was compared to Day 1 measurements where the rats were injected with aCSF as the control.

Upon analyzing the stained coronal slices following the experiment, the placement of the cannula was found to be more medial than expected. Therefore, the stereotaxic measurements of coordinates may have been erroneous due to machinery malfunction during surgery.

Conclusion:

Despite the preliminary study showing that DPDPE trended towards producing an increase in feeding at 180 minutes post-injection, this trend was not seen in the replication study testing DPDPE and U50488 over a range of doses. This suggests that kappa- and delta-opioid receptors are unlikely to be a significant modulatory factor of morphine-elicited eating in the LS. Thus, my evidence supports the hypothesis that mu-opioid receptors are the main opioid receptor subtype mediating feeding behavior in the lateral septum.

Kappa and/or delta-opioid receptors in the LS might mediate feeding suppression and future studies can be conducted to test this possibility. Old studies have suggested that kappa opiates may have inhibitory effects to mu opiates on motor behavior and fluid regulation (Mansour, 1988). Therefore, we could determine whether LS kappa- and delta-opioid receptor activation in the LS might decrease feeding behavior. This can be determined in a food deprivation experiment where rats are given access to only water but no food for 72 hours. The food intake will then be measured to assess if the rats eat less when given agonist for their respective opioid receptor. Another possible future project could include gathering immunohistochemistry data on the LS neural connections and synaptic activity analyzed by C-Fos staining. Further research of the lateral septum along with its cognate projections to other brain regions may elucidate its neural implication in feeding. This could eventually help with future therapeutic interventions within these regions for treatment of obesity and eating disorders.

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