Oxytocin Receptor Neuron Influence on Eating Behavior

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Introduction

- Oxytocin (OXT) is a hypothalamic neuropeptide known to contribute to a multitude of different physiological and social behaviors.
- In regards to eating behavior, OXT has been shown to play a role in the control of food intake, specifically, meal cessation.
- OXT neurons achieve this in part through their projections to brain regions involved in reward signaling, most prominently the ventral tegmental area (VTA) (Xiao et al., 2017). The VTA is a brain region that is known to play a major role in motivated behavior and addiction (Trutti et al., 2019).
- The surrounding literature suggest that OXT projections to the VTA act on OXT receptor-expressing neurons that will eventually promote satiation and lead to earlier meal cessation.
- Goal of experiment: investigate the effect of OXTR neuron activity on food intake.
- We utilized transgenic mice and chemogenetics to allow for the targeting and activation of OXTR neurons within our model organism in order to measure the subsequent effects this activation has on eating behavior.
- Transgenic mice: genetically modified organisms; the mice in this study have been genetically modified to express Cre recombinase in OXTR neurons.
- The presence of Cre recombinase in these neurons allowed us to target them via a Cre-dependent adeno-associated virus (AAV).
- Chemogenetics: insertion of engineered receptors into OXTR neurons in the VTA is known as a Designer Receptors Exclusively Activated by Designer Drugs (DREADD). The DREADD we used in this experiment is hM3D(Gq), an excitatory g-protein coupled receptor.
- These excitatory DREADDs are activated by synthetic drug clozapine-N-oxide (CNO).
- Hypothesis: chemogenetic activation of OXTR neurons will result in a reduction of chow intake in mice.

Methods

Subjects

- 14 naive mice (7 males, 7 females) expressing the genotype OXTR-Cre were individually housed in a BioDAQ system where eating behaviors were monitored.
- The mice were maintained on a 12-12 light/dark cycle and administered a standard chow diet.

Virus Injection Surgery

- Mice underwent stereotaxic brain surgery during which the AAV was injected into the VTA.
- The virus we injected into the VTA is called AAV1-hSyn-DIO-hM3D(Gq)-mCherry.

Experimental Treatment Days

- This experiment measured food intake under two different treatment conditions: saline and CNO.
- There were 2 experimental days separated by 48 hours. All mice received both the saline and CNO treatments on different days and in a counterbalanced order.

Figure 1

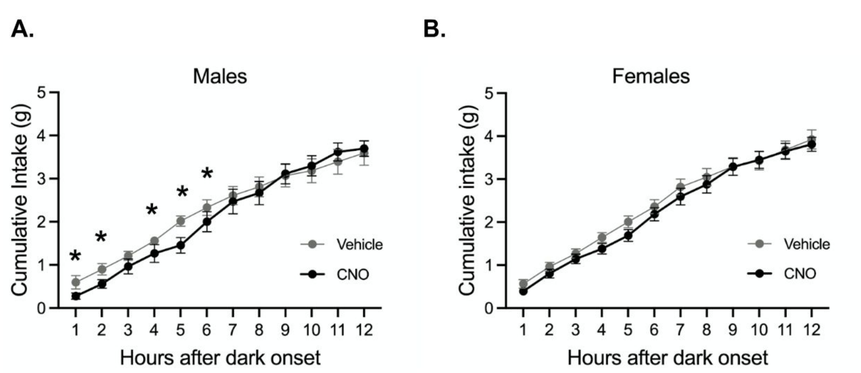

Fig 1. Cumulative intake (g) over 12 hours following dark onset in male and female subjects. In males (A), CNO administration significantly reduced cumulative intake compared to the vehicle at multiple time points (indicated by *), particularly during the initial hours. In females (B), no significant difference in cumulative intake was observed between vehicle and CNO conditions.

Figure 2

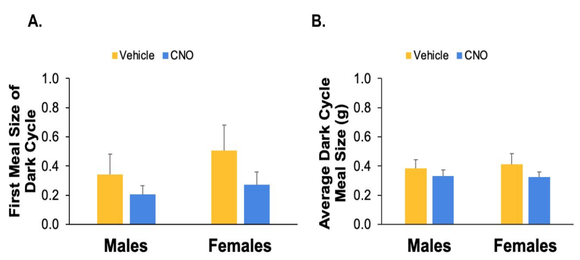

Fig 2. Meal pattern analysis in male and female subjects treated with vehicle or CNO. (A) First meal size of dark cycle(g) and (B) average dark cycle meal size for each group. No significant differences were observed between vehicle and CNO treatment for first meal size of the dark cycle and average dark cycle meal size in either sex. However, a trend towards a decreased first meal size of dark cycle was observed in CNO-treated subjects compared to vehicle-treated subjects.

Figure 3

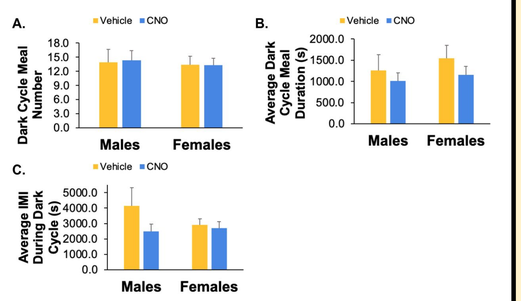

Fig 3. Additional meal pattern analysis in male and female subjects treated with vehicle or CNO. (A) Dark cycle meal number, (B) average dark cycle meal duration (s), and (C) average inter-meal interval (IMI) are shown for each group. No significant differences were observed between vehicle and CNO treatments for either sex.

References

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Trutti, A. C., Mulder, M. J., Hommel, B., & Forstmann, B. U. (2019). Functional neuroanatomical review of the ventral tegmental area. NeuroImage, 191, 258–268.
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Results

- The effects of CNO on cumulative hourly dark phase chow intake were assessed by ANOVA.
- A three-way ANOVA showed a significant interaction between time, sex and CNO ($F(12,204) = 2.82$, $p < 0.05$), so we further analyzed each sex separately.
- In male mice, a two-way ANOVA showed a significant interaction between time and CNO ($F(12,96) = 2.86$, $p = < 0.05$).
- In males, planned pairwise comparisons showed that CNO significantly suppressed food intake relative to vehicle at hours 1, 2, 4, 5, and 6 in the dark cycle.
- In females, a two-way ANOVA showed a significant effect of time ($F(12,108) = 88.5$, $p < 0.05$), however, there was no significant effect of CNO nor an interaction between time and CNO.
- Two-way ANOVAs including males and females found no significant effects of sex or CNO treatment on 20-h cumulative intake, number of meals in the dark phase, average inter-meal interval, and average meal size in the dark phase.
- There was a trend toward a significant effect of CNO to reduce the size of the first meal of the dark phase, but this did not reach significance ($F(1,17) = 4.135$, $p = 0.058$)

Discussion

- The results revealed that CNO significantly reduced chow intake during the dark cycle in male mice.
- The effect in male mice occurred only during the the first half of the dark cycle, and this is consistent with the reported CNO half-life of several hours and electrophysiological data suggesting that CNO can affect activity of DREADD-expressing neurons for up to 9 hours after treatment (Guettier et al., 2009).
- The lack of effect of CNO in female mice is consistent with previous reports of sex differences in the feeding effects of oxytocin.
- We found no effects of CNO on any of the meal pattern variables we assessed.
- We expected that activation of VTA OXT receptor neurons would suppress the size of the first meal of the dark phase, based on previous studies in rats (Mullis, 2013), however, we observed only a trend toward reduced first meal size that did not reach significance.
- We also expected to see a significant reduction in average meal size during the dark phase in male mice that showed a suppression of food intake.
- It is possible that we did not find effects on meal pattern variables because the effect on chow intake in male mice was relatively small.
- Overall, the results from this experiment suggest that the activation of OXTR neurons in the VTA decreases food intake in male mice.
- This provides further support that OXT's anorexigenic
- effects are mediated in part by the activation of OXTR neurons in the VTA.