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The Effects of Sub-Chronic Olanzapine Treatment on Sucrose Preference in Mice

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by

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Abstract

Adults are often prescribed psychiatric medications for a variety of FDA-approved indications as well as for "off-label" indications. The short- and long-term effects of psychiatric medication exposure are not well known. Second-generation antipsychotic (SGA) medications induce weight gain possibly by increasing consumption of high-fat and high-sugar foods, but the mechanisms underlying weight gain are still unclear. To investigate whether SGA medication exposure induces a preference for palatable foods, this experiment examined the effect of chronically administered olanzapine (1, 3, and 6 mg/kg) on sucrose preference using a two-bottle preference test in a cohort of eight mice. The mice were allowed to freely consume water and a sucrose solution in their home cage and the bottles were weighed daily. Sucrose preference was first tested using a 0.25% sucrose solution and subsequently using 2% sucrose solution. Once preference for sucrose was determined under the vehicle and treatment conditions using both sucrose concentrations, a multilevel linear regression was used to analyze the data. The analysis revealed changes, relative to baseline, in preference for the 0.25% sucrose solution during vehicle and olanzapine administration periods, without a clear differentiation in preference between the two conditions. When 2% sucrose was used, preference was higher than with 0.25% as expected and there were no systematic changes in preferences during vehicle and olanzapine administration periods. Total liquid consumption, relative to baseline, decreased following both vehicle and olanzapine administration. Olanzapine administration appeared to induce increases in food grinding. Finally, there was no effect of olanzapine on weight gain. In summary, the current results do not indicate that olanzapine increases preference for obesogenic foods like sucrose, but that interpretation is limited by the absence of weight-increasing effects of

olanzapine, leaving open the possibility that when weight gain occurs, it is driven, at least in part, by increased preference for obesogenic foods.

The Effects of Sub-Chronic Olanzapine Treatment on Sucrose Preference in Mice

Antipsychotic medications are commonly used to treat disorders like schizophrenia, certain forms of bipolar disorder, and severe depression (Christian et al., 2012). There are two classes of antipsychotic medications – first- and second-generation antipsychotics. First generation antipsychotics (FGAs) are D2 receptor antagonists and carry risk for developing extrapyramidal symptoms (EPS) such as tardive dyskinesia, Parkinsonism, akathisia, and acute dystonia. Second generation antipsychotics (SGAs) are also D2 receptor antagonists, but they differ from FGAs because they carry less risk of EPS, potentially due to differences in their binding affinity for D2 receptors and their binding affinity for 5-HT2A receptors (Tarsy et al., 2002).

Although SGAs are associated with a reduced risk of EPS, they have been shown to produce metabolic disturbances and weight gain possibly by increasing consumption of high-fat and high-sugar foods, but the mechanisms underlying weight gain are still unclear. Obesity is twice as prevalent in patients with schizophrenia who are taking SGAs as compared to the general population (Wirshing, 2004). The weight gain could be caused by lifestyle factors of these patients, genetic factors, their demographics, and problems with regulation in the endocrine system, among other factors. Importantly, adverse side effects of medication can promote noncompliance with the medication regimen and produce poor self-esteem.

In human studies, the literature shows that there is an increase in overall food intake, specifically a greater intake of high fat foods, when patients are taking SGAs. Overall caloric intake in patients taking olanzapine significantly increased as compared to patients taking haloperidol, a first-generation antipsychotic (Gothelf et al., 2002). A predilection for palatable

food, like fast food and other high fat foods, seems to be a potential cause of obesity in patients taking SGAs (Elman, Borsook, & Lukas, 2006). Patients treated with SGAs consumed more fat, saturated fat, and proteins, but less carbohydrates compared to control participants (Cuerda et al., 2014). If SGAs increase weight gain, changes in food preference and/or overall food consumption could mediate or exacerbate the weight gain and metabolic effects.

The possible effect of SGAs on satiation levels, or the ability to feel full and satisfied, is another factor that could play an important role in both food consumption and weight gain. Hunger levels, and therefore satiation, are determined by hormones called ghrelin and leptin. Ghrelin is a hormone that increases appetite, and leptin is a hormone that decreases appetite. These two hormones work together to maintain homeostasis and body weight regulation by telling the body when it needs to eat and when it should stop eating through the feelings of hunger and satiation. Olanzapine may increase the amount of ghrelin circulating in the body which would cause patients to feel hungry more often and would make it more difficult for them to feel satiated (Murashita et al., 2005). Consistent with this suggestion, one study found that after eating a standardized breakfast, the group being treated with SGAs reported significantly higher hunger levels and significantly lower satiation levels than the control group (Cuerda et al., 2014).

Non-human animal studies are important for investigating the effects of SGAs on weight gain because of the similarities in both neurological and metabolic function and because rigorously controlled studies of doses and duration of exposure are exceedingly difficult in humans. Sex is a common variable considered in animal studies because of the natural differences in feeding patterns and weight between male and female animals. Female rats treated

with olanzapine experienced an increase in food intake and body weight, whereas male rats did not exhibit the same effects (Choi et al., 2007). In another experiment examining food consumption in female rats, olanzapine increased food intake, meal size, and body weight which is likely due to an olanzapine-induced state of hyperphagia creating an inability to regulate satiation levels (Davoodi et al., 2009).

Unlike human studies, animal studies allow more control over the measurement of food preference and motivation for unhealthy foods. A preference for palatable foods has also been found in animal studies when animals have been given the option to eat high fat/high sugar foods. One study found that animals being fed a high fat/high sugar diet while on olanzapine grew faster than the control mice and mice treated with olanzapine also exhibited a strong preference for high fat/high sugar foods (Smith, Vickers, & Shepherd, 2011). Another study that looked at motivation to work for palatable food found that olanzapine increased responding reinforced by to sucrose which supports the idea that olanzapine may increase motivation to work for palatable food (van der Zwaal et al., 2012). Finally, another study examining the acute effects of a D2 receptor antagonist on sucrose preference in rats found a decrease in sucrose preference when given a low concentration of sucrose, but significantly increased overall sucrose intake at a higher concentration (Muscat & Willner, 1989).

Overall, the literature does not point to one consistent answer regarding the effects of SGAs on various mechanisms underlying weight gain in both humans in mice. It is unlikely that there is only one factor solely responsible for all of these effects as opposed to a complex interaction of different factors such as the environment, genetics, and biochemical changes. The effects appear to depend on the dose given, the sex of the subject, and the diet that the subject is

on while taking the medication. Overall, the literature indicates SGAs affect the motivation to work for sucrose, but there seems to be a gap in the literature studying preference for sucrose and sucrose consumption when it is being freely offered in the home cage.

Based on results found in the current literature on antipsychotics, this study aims to measure the effect of olanzapine on four different variables – sucrose preference, total liquid consumption, food consumption, and body weights. It is hypothesized that olanzapine will increase preference for sucrose over water, and that preference will systematically change as the dose increases. It is also predicted that olanzapine will have an effect on total liquid consumption. Finally, it is expected that olanzapine will increase both food consumption and body weights over the course of the study.

Methods

Subjects

A group of four male and four female C57BL6/J mice (Jackson Laboratory, Bar Harbor, ME) were used for this experiment. At the start of the experiment, the mice were 8 weeks old. They were housed in standard cages and fed standard lab chow under a 12-hour light/dark cycle. Chow was freely available in the home cage throughout the duration of the study. All methods were performed within the guidelines of the Institutional Animal Care and Use Committee of Pennington Biomedical Research Center.

Materials

For this study, 18 conical tubes were used to supply the water and sucrose solutions in each of the eight cages in addition to the drip cage. A scale was used to measure body, food, and

bottle weights each day. For the drug dough, Betty Crocker Sugar Cookie Mix (General Mills, Inc, Minneapolis, MN) was combined with Eggbeaters (Con Agra Foods, Inc. Chicago, IL), unsalted Land O' Lakes Sweet Cream Butter (Land O' Lakes, Inc. Arden Hills, MN), and a stock mixture of cookie mix containing olanzapine using a KitchenAid hand mixer. For the sucrose solutions, sugar was mixed into warm tap water. Depending on what the desired yielded total of the solution was, the amount of sugar mixed into the water was adjusted to fit the 0.25% and 2% set concentrations. The dough mixture and sucrose solutions were stored in glass bottles in a refrigerator controlled for temperature.

Procedure

Two-Bottle Choice Procedure

A two-bottle choice procedure was used for the assessment of sucrose preference (Eagle et al., 2016). As shown in Figures 1-4, the entire procedure was completed twice – once with a 0.25% sucrose solution for phase 1 and then a 2% sucrose solution for phase 2. Prior to phase 1, two 50 ml conical tubes both filled with water were placed side-by-side in each cage to determine the baseline water consumption of each mouse for four days. After the initial four-day water consumption baseline, one of the two tubes was removed, and the water was replaced with a 0.25% sucrose solution. Each of the remaining water tubes (one per cage) were labeled with an "B" and all of the sucrose-filled tubes were labeled with a "A" for identification purposes. Both tubes in each cage were measured daily to assess sucrose preference, calculated as the grams of sucrose consumed relative to the grams of sucrose solution plus water (total grams of liquid) consumed. The position of the bottles was switched in the cage after weighing daily to control for an effect of the position of the bottle. An empty cage ("drip cage") that had both the water

and sucrose tubes in it followed the same procedure as the other cages to measure and control for loss of liquid through dripping.

Oral Self-Dosing

Olanzapine is the second-generation antipsychotic medication chosen for this study. The doses of olanzapine used were 1, 3, and 6 mg/kg. The vehicle used to self-administer the drug was sugar cookie dough. Fixed proportions of each ingredient were used to create the drug dough by mixing the olanzapine thoroughly with the plain cookie dough powder first and then adding in wet ingredients (butter and Egg Beaters). Plain dough was made using the following proportions – 0.74 of Sugar Cookie mix, 0.17 of butter, and 0.09 of Egg Beaters. Drug dough was created using the same proportions, but with olanzapine mixed into the dry ingredients. Dough was always administered at 12.5 grams of dough per kilogram of body weight. For 1, 3, and 6 mg/kg doses of olanzapine, concentrations of 0.08, 0.24, and 0.48 mg olanzapine per gram of dough were used, respectively. Once the mixture was created, it was stored in a refrigerator for no more than seven days. Using the daily body weights of the mice, they were given the appropriate amount of drug dough in their cage to self-administer. This procedure was selected to intentionally reflect the self-dosing practices in human clinical populations.

Drug Treatment Schedule

In an attempt to control for any confounds due to time, the mice were divided into four paired groups and the dosing schedules of each group were staggered so that changes in outcome variables, if observed, could more confidently be attributed to olanzapine administration. Each pair consisted of one male mouse and one female mouse. All eight of the mice followed the same

sequence of phases, but on different post-natal days (PND). The timelines for each pair of mice can be seen in Figures 1-4 below.

Figure 1

Phase 1 and 2 Treatment Timeline for MG01 and MG05

Phase:	Phase 0: Water vs. Water							
PND:	76-94							
Dose:	No Treatment							
Phase:	Phase 1: Water vs. 0.25% Sucrose							
PND:	95-99	100-111	112-120	121-132	133-139	140-151	152-169	170-174
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment
Phase:	Phase 2: Water vs. 2% Sucrose							
PND:	175-181	182-192	193-197	198-209	210-216	217-227	228-232	233-241
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment

Figure 2

Phase 1 and 2 Treatment Timeline for MG02 and MG06

Phase:	Phase 0: Water vs. Water							
PND:	76-94							
Dose:	No Treatment							
Phase:	Phase 1: Water vs. 0.25% Sucrose							
PND:	95-105	106-116	117-132	133-146	147-153	154-167	168-174	175-179
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment
Phase:	Phase 2: Water vs. 2% Sucrose							
PND:	180-185	186-197	198-203	204-214	215-219	220-230	231-235	236-241
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment

Figure 3

Phase 1 and 2 Treatment Timeline for MG03 and MG07

Phase:	Phase 0: Water vs. Water							
PND:	76-94							
Dose:	No Treatment							
Phase:	Phase 1: Water vs. 0.25% Sucrose							
PND:	95-111	112-132	133-139	140-151	152-167	168-178	179-185	186-189
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment
Phase:	Phase 2: Water vs. 2% Sucrose							
PND:	190-195	196-206	207-211	212-223	224-229	230-240	241-245	245-250
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment

Figure 4

Phase 1 and 2 Treatment Timeline for MG04 and MG08

Phase:	Phase 0: Water vs. Water							
PND:	76-94							
Dose:	No Treatment							
Phase:	Phase 1: Water vs. 0.25% Sucrose							
PND:	95-116	117-132	133-137	138-148	149-157	158-168	169-174	175-179
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment
Phase:	Phase 2: Water vs. 2% Sucrose							
PND:	180-185	186-197	198-203	204-214	215-219	220-230	231-235	236-241
Dose:	Vehicle	Drug Dough (1 mg/kg)	Vehicle	Drug Dough (3 mg/kg)	Vehicle	Drug Dough (6 mg/kg)	Vehicle	No Treatment

Data Analysis

The effects of sub-chronic olanzapine dosing on sucrose preference, total grams of liquid consumed, food consumption, and body weights for each phase were analyzed using a multilevel linear regression analysis in RStudio (R Core Team, 2018) with the lmerTest function of the lmerTest Package, which uses Satterthwaite degrees of freedom to provide p-values for regression coefficients (Kuznetsova, Brockhoff, & Christensen, 2017). Sucrose preference was calculated by dividing the grams of sucrose solution (bottle A) consumed by the sum of the total grams consumed from both bottles. Total grams consumed was calculated by summing the amount consumed from each bottle. Food consumed was calculated by subtracting the current

day's food weight from the prior day's food weight. If more than one day intervened between two food weights, the difference between the two food weights was divided by the number of days intervened to calculate the average daily consumption. The multilevel linear model analyzed the fixed effects of the different treatment levels (each vehicle and olanzapine administration period were treated as a different level) on the dependent variables and compared the values to the initial "No Treatment" period at the start of each phase (0.25% or 2% sucrose). If there was no effect of the vehicle (cookie dough) by itself, the initial vehicle condition should not deviate from the "No Treatment" control condition. Sucrose preference, total grams of liquid consumed, and total food consumed were analyzed with a model that included a fixed effects predictor of Treatment Number (each vehicle and olanzapine administration period following the initial No Treatment period incremented the Treatment Number variable) and a random effects intercept for each mouse. The analysis for body weights also included the "Phase Day Number" as a fixed effects predictor to check for any main effects or interactions of natural growth on body weights.

Results

Sucrose Preference

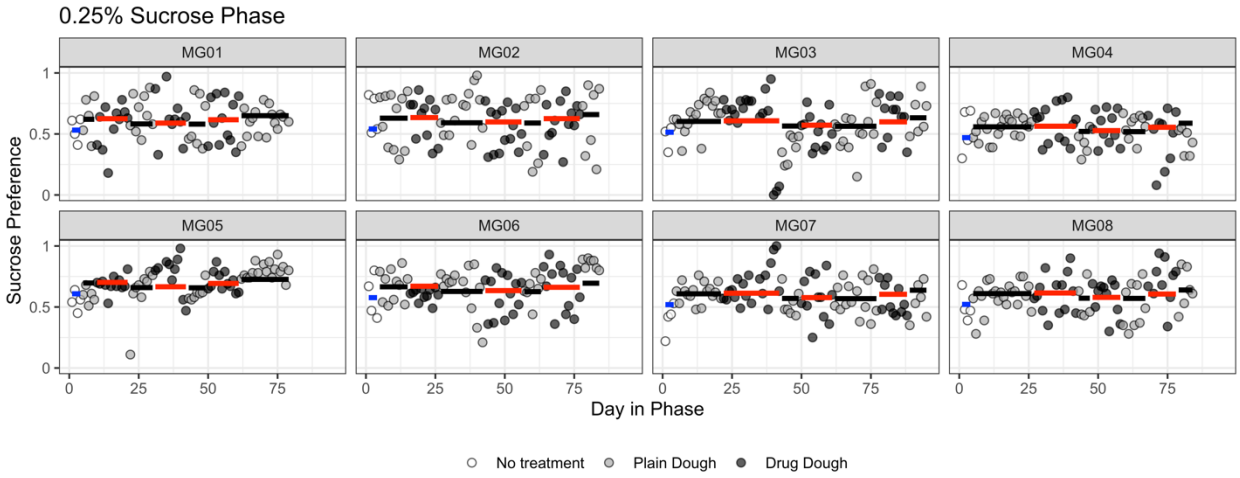
Although the computational analysis in RStudio did not include statistical comparison between the two phases, there was a large difference between overall sucrose preference in phase 1 and phase 2. As shown in Table 1 and Figure 5, the y-intercept of 0.535 indicates that there was only a 53.5% preference for the 0.25% sucrose solution over water whereas Table 2 and Figure 6 show that sucrose preference was approximately 92.9% for 2% sucrose over water

during phase 2. This is consistent with much previous research indicating increased preference for higher sucrose concentrations.

During phase 1, administration of olanzapine doses of 1 and 6 mg/kg was associated with increased sucrose preference compared to the control "No Treatment" condition but interpreting this change in sucrose preference as an effect of olanzapine is confounded by similar magnitude increases in sucrose preference during the first and last vehicle administration periods (see Table 1 and Figure 5).

Figure 5

Phase 1 Sucrose Preference Multilevel Linear Regression Graph



Note. The blue lines indicate the "No Treatment" condition. The black lines indicate the "Plain Dough" condition where the mice were given vehicle. The red lines indicate the "Drug Dough" conditions where the mice were being dosed with 1, 3, and 6 mg/kg of olanzapine, respectively.

Table 1

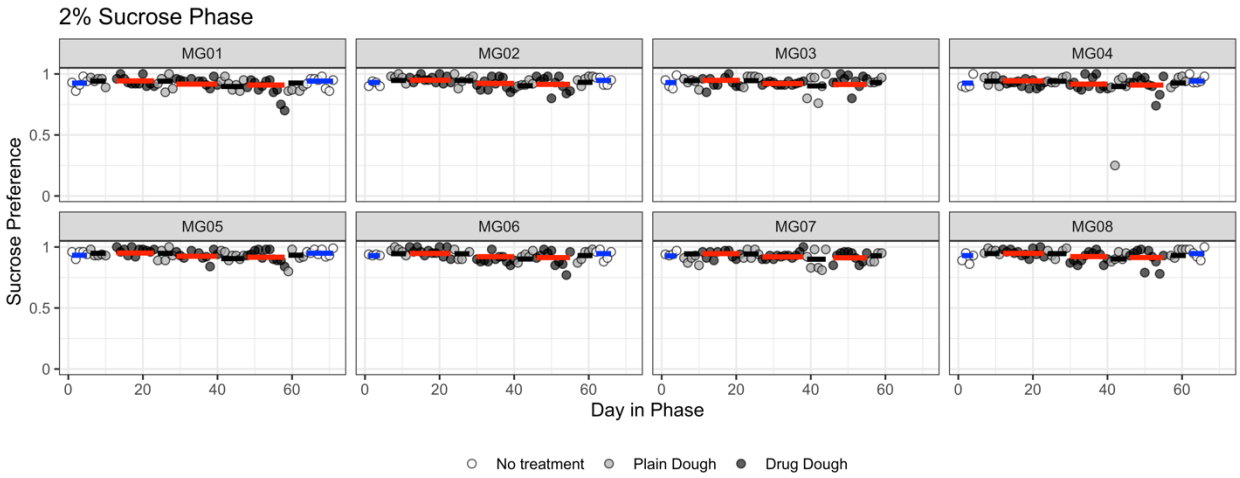
Phase 1 (0.25%) Sucrose Preference Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	0.535	0.03	82.97	16.51	< .001
Vehicle	0.089	0.03	642.50	2.75	.006
1 mg/kg Dose	0.094	0.03	641.23	2.92	.004
Vehicle	0.051	0.03	641.77	1.48	.138
3 mg/kg Dose	0.058	0.03	641.00	1.76	.079
Vehicle	0.049	0.03	641.56	1.45	.146
6 mg/kg Dose	0.085	0.03	640.95	2.59	.010
Vehicle	0.118	0.03	642.56	3.46	< .001

Phase 2 results were much less variable because of how high preference was overall during this phase. Because preference for the 2% sucrose solution was already almost exclusive during the initial “No Treatment” baseline condition, there was no room for preference to increase significantly from an already exclusive preference. Although there was a statistically significant decline in sucrose preference during the third vehicle administration period, this was the only deviation from the control condition that met criteria for statistical significance.

Figure 6

Phase 2 Sucrose Preference Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 2

Phase 2 (2%) Sucrose Preference Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	0.929	0.01	326.70	99.82	< .001
Vehicle	0.016	0.01	475.60	1.30	.193
1 mg/kg Dose	0.018	0.01	475.90	1.70	.090
Vehicle	0.016	0.01	475.70	1.25	.214
3 mg/kg Dose	-0.008	0.01	476.10	-0.76	.448
Vehicle	-0.027	0.01	475.50	-2.27	.024
6 mg/kg Dose	-0.016	0.01	475.50	-1.46	.146
Vehicle	0.001	0.01	476.10	0.05	.960
No Treatment	0.017	0.01	480.80	1.31	.192

Total Liquid Consumed

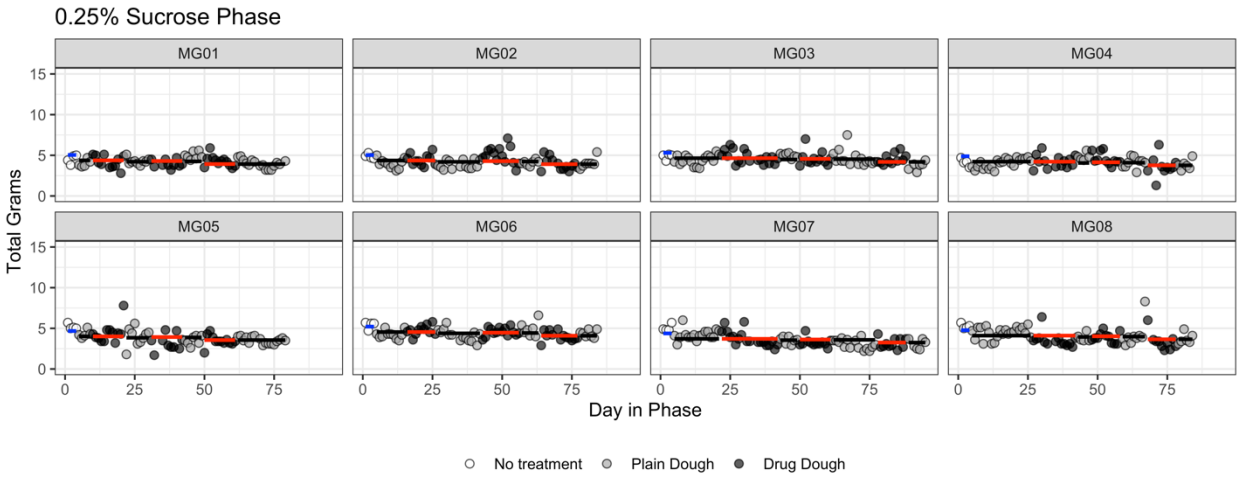
Aside from olanzapine affecting preference for sucrose over water, the data were also analyzed to look for an effect of olanzapine on total liquid consumed. At the beginning of Phase

1, mice consumed approximately 4.9 grams of fluid per day. Subsequent vehicle and olanzapine administration periods were associated with reductions in total liquid consumption between approximately 0.7 and 1.1 g (Table 3 and Figure 7). Because both vehicle and olanzapine administration periods were associated with reductions of equivalent magnitude, there is little basis for concluding an effect of olanzapine on total liquid consumed. Rather, it may have been that cookie dough consumption itself reduced total liquid consumed.

During phase 2, total liquid consumed was approximately 6.8 g per day during the initial No Treatment period, approximately 1.9 g more per day than in Phase 1. Similar to what was observed during Phase 1, total consumption was lower during all vehicle and olanzapine administration periods (although the difference was not statistically significant during the first vehicle administration period; Table 4 and Figure 8). Interestingly, when the mice stopped receiving plain dough and returned to a “No Treatment” condition at the end of phase 2, their consumption did increase from 5.25 to 6.13. This suggests that there might be an effect of the dough itself on thirst levels as measured by their liquid consumption.

Figure 7

Phase 1 Total Liquid Consumed Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

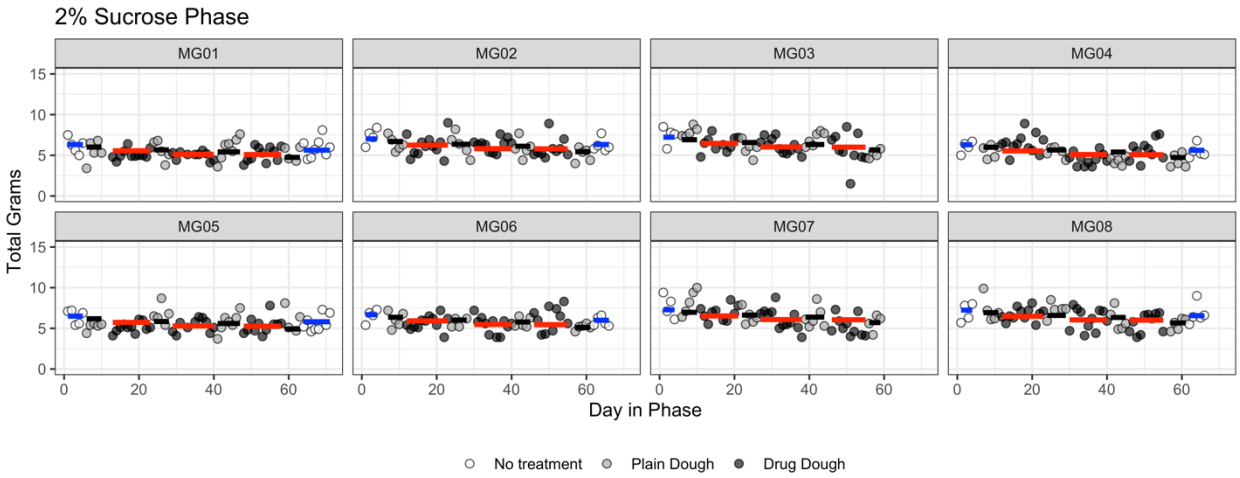
Table 3

Phase 1 (0.25%) Total Liquid Consumed Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	4.913	0.18	37.21	27.96	< .001
Vehicle	-0.666	0.16	641.90	-4.30	< .001
1 mg/kg Dose	-0.666	0.15	641.24	-4.31	< .001
Vehicle	-0.830	0.16	641.52	-5.05	< .001
3 mg/kg Dose	-0.746	0.16	641.12	-4.74	< .001
Vehicle	-0.790	0.16	641.41	-4.86	< .001
6 mg/kg Dose	-1.120	0.16	641.10	-7.10	< .001
Vehicle	-1.121	0.16	641.93	-6.82	< .001

Figure 8

Phase 2 Total Liquid Consumed Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 4

Phase 2 (2%) Total Liquid Consumed Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	6.821	0.24	33.79	27.94	< .001
Vehicle	-0.314	0.26	475.01	-1.23	.220
1 mg/kg Dose	-0.762	0.22	475.06	-3.46	< .001
Vehicle	-0.656	0.26	475.02	-2.55	.011
3 mg/kg Dose	-1.202	0.22	475.09	-5.44	< .001
Vehicle	-0.900	0.25	475.00	-3.64	< .001
6 mg/kg Dose	-1.228	0.22	475.00	-5.50	< .001
Vehicle	-1.576	0.26	475.08	-6.02	< .001
No Treatment	-0.688	0.26	476.08	-2.61	.009

Food Consumption

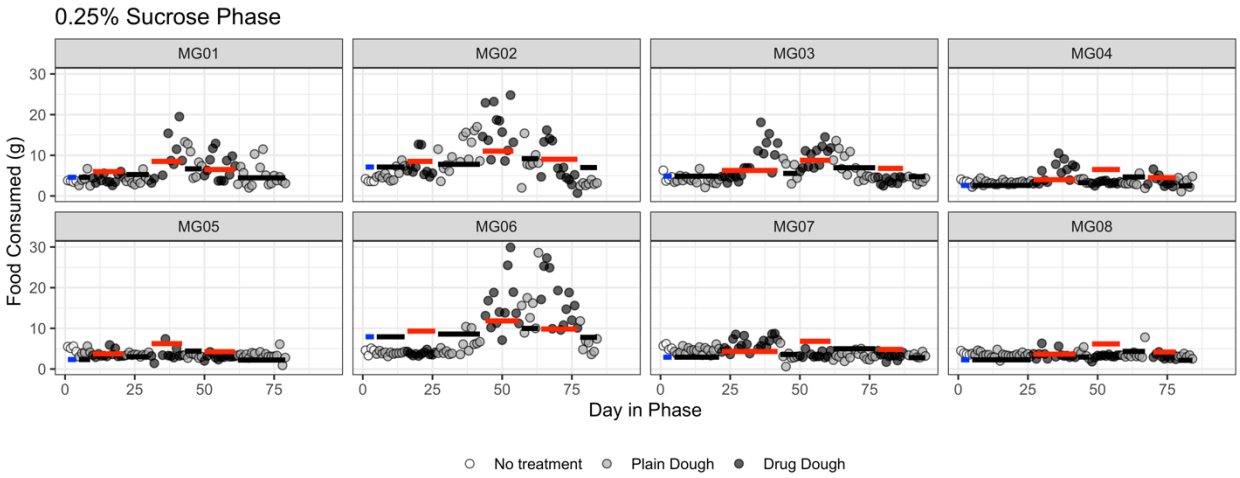
Phase 1 and phase 2 both began with a similar amount of food consumed daily as seen in Tables 5 and 6 during the “No Treatment” condition. There was a lot of variability in the amount

of food the mice ate across all of the conditions due to a behavior known as “food grinding.” “Food grinding” is when the mice chew large amounts of their food, but instead of consuming it, they spit it back into their cages. So, when weighing the food, it can appear that a specific mouse consumed over 20 grams of food overnight, when in reality, most of the missing food is sitting in their cages in broken pieces. It is unclear whether food grinding is caused by stress, hunger, boredom, or any number of other causes. It does appear that the mice engaged in food grinding behaviors more often in phase 1 than in phase 2 as shown by the spikes in consumption in Figures 9 and 10. The mice demonstrated an increase in food consumption as compared to baseline during phase 1, but in phase 2 there was a general decrease in consumption. It also appears that food grinding was more common during the latter half of each phase when the doses were higher, but then eating habits returned to normal after the drug was discontinued.

During phase 1, food consumption significantly increased from baseline consumption in all three drug conditions. During each vehicle condition, consumption decreased from the drug treatment amount that preceded it but did not return to baseline until the final vehicle condition. Throughout phase 1, a pattern was observed that showed a significant increase in consumption while the mice were being dosed, but then a decrease in consumption each time the mice were taken off of the drug. Food consumption peaked during the 3 mg/kg dose and steadily declined back to baseline until the end of phase 1 as shown in Table 5.

Figure 9

Phase 1 Food Consumption Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 5

Phase 1 (0.25%) Food Consumption Multilevel Linear Regression Results

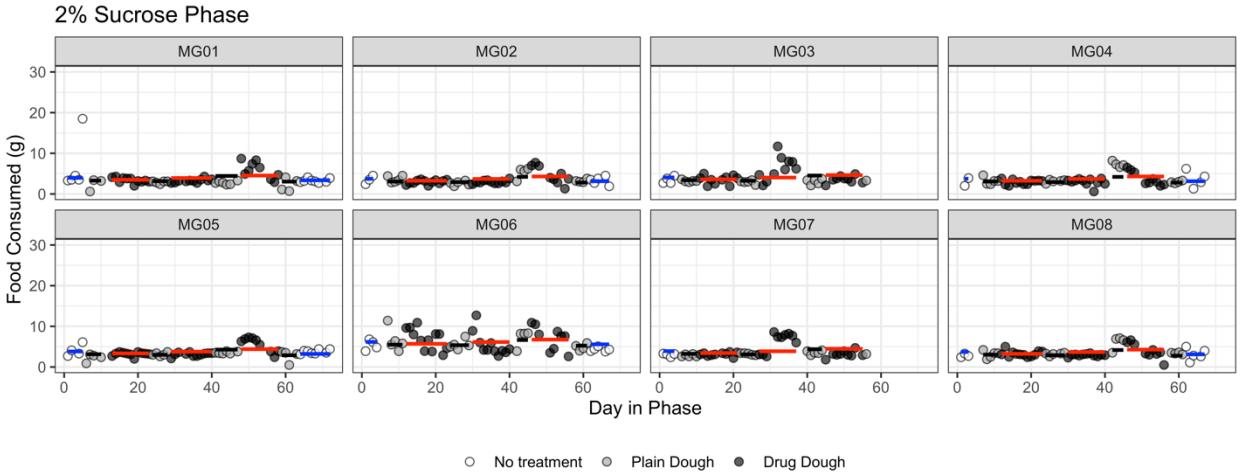
Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	4.325	1.00	16.49	4.33	< .001
Vehicle	-0.002	0.69	630.35	0.00	.997
1 mg/kg Dose	1.395	0.69	630.07	2.03	.043
Vehicle	0.674	0.74	630.22	0.91	.361
3 mg/kg Dose	3.906	0.71	630.01	5.52	< .001
Vehicle	2.070	0.73	630.14	2.85	.004
6 mg/kg Dose	1.909	0.71	630.02	2.70	.007
Vehicle	-0.127	0.73	630.38	-0.17	.863

Unlike phase 1, the mice demonstrated a general decrease in food consumption for most of phase 2. It is possible that the increase in liquid intake during the 2% sucrose phase interfered with food consumption. Even though a decrease in consumption was observed, none of the deviations were significantly different from the baseline. There was a slight increase in

consumption across the mice immediately prior to and during the 6 mg/kg condition, but the increase during the last vehicle condition was the only significant difference. It is possible that the effects of the higher dose prompted food grinding behaviors or induced an overall increase in hunger towards the end of phase 2.

Figure 10

Phase 2 Food Consumption Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 6

Phase 2 (2%) Food Consumption Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	4.139	0.44	26.60	9.39	< .001
Vehicle	-0.674	0.43	437.28	-1.58	.114
1 mg/kg Dose	-0.474	0.37	437.27	-1.29	.197
Vehicle	-0.812	0.42	437.16	-1.94	.053
3 mg/kg Dose	-0.038	0.37	437.22	-0.10	.918
Vehicle	0.463	0.42	437.09	1.10	.274
6 mg/kg Dose	0.571	0.38	437.19	1.51	.131
Vehicle	-0.927	0.46	437.59	-2.00	.047
No Treatment	-0.599	0.43	438.08	-1.38	.168

Results for the phase 2 food consumption data appear to be skewed by an outlier from MG01 in the initial "No Treatment" phase (see Figure 10). It is likely that the mouse was exhibiting food grinding behaviors, but since the point appears to be an isolated occurrence, an additional analysis was done with the point excluded from the data. When this point was excluded, all of the significance values and intercepts for the other conditions were affected. Without the outlier, consumption during the "No Treatment" phase decreases to a starting point of 3.62. This change caused the 6 mg/kg condition and the vehicle condition preceding it to become significantly greater than baseline.

Table 7

Phase 2 (2%) Food Consumption Multilevel Linear Regression Results Excluding Outlier

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	3.622	0.43	22.57	8.45	< .001
Vehicle	-0.167	0.39	436.17	-0.43	.670
1 mg/kg Dose	0.043	0.34	436.19	0.13	.898
Vehicle	-0.294	0.38	436.11	-0.77	.445
3 mg/kg Dose	0.480	0.34	436.15	1.41	.158
Vehicle	0.988	0.39	436.08	2.54	.011
6 mg/kg Dose	1.088	0.35	436.12	3.13	.002
Vehicle	-0.402	0.43	436.49	-0.94	.347
No Treatment	-0.056	0.40	436.94	-0.14	.889

Body Weights

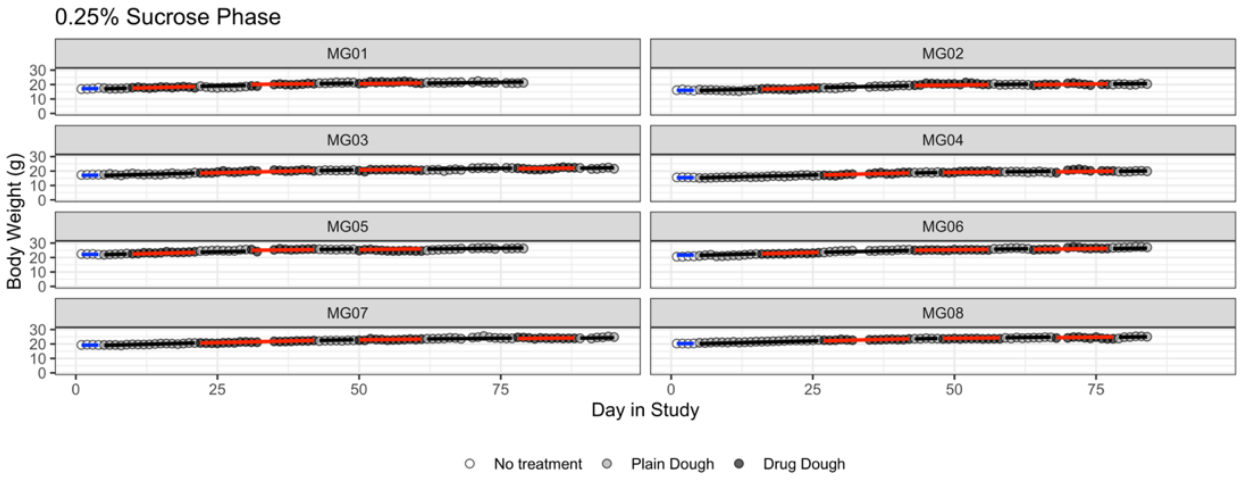
The body weight analysis included both treatment and day in phase as predictors for body weight. Since it was expected that there would be a natural progression of body weight over time due to the natural growth and development of the mice, "Phase Day Number" was included in the analysis to check for any effects. Although there was a gradual increase in body weights over

the course of the two phases, there does not appear to be a clear drug effect on the body weights. The progression of body weights can be seen below in Figure 11 and Figure 12.

In phase 1, body weights did significantly increase between the 3 and 6 mg/kg treatments, but these effects were observed in both drug and vehicle conditions which makes it difficult to determine whether it was due to any effects of the drug. When phase day number was added to the analysis, none of the conditions showed significant effects as seen in Table 8.

Figure 11

Phase 1 Body Weights Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 8

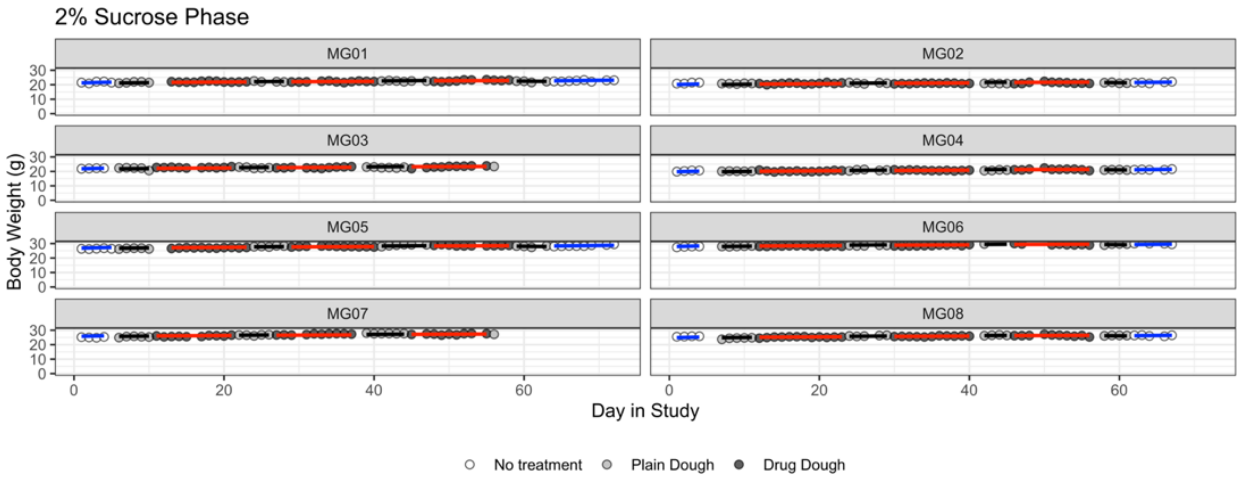
Phase 1 (0.25%) Body Weights Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	18.581	0.94	7.83	19.78	< .001
Vehicle	-0.570	0.25	630.00	-2.27	.023
1 mg/kg Dose	-0.523	0.28	630.14	-1.88	.061
Vehicle	-0.113	0.37	630.24	-0.30	.762
3 mg/kg Dose	1.663	0.45	630.33	3.69	< .001
Vehicle	1.714	0.51	630.30	3.38	< .001
6 mg/kg Dose	1.182	0.48	630.39	2.48	.013
Vehicle	0.919	0.65	630.41	1.41	.160
No Treatment Slope	0.021	0.08	630.00	0.26	.792
Vehicle Slope	0.077	0.08	630.00	0.95	.344
1 mg/kg Slope	0.070	0.08	630.00	0.87	.385
Vehicle Slope	0.058	0.08	630.00	0.72	.474
3 mg/kg Slope	0.020	0.08	630.00	0.24	.808
Vehicle Slope	0.020	0.08	630.00	0.25	.802
6 mg/kg Slope	0.023	0.08	630.00	0.28	.780
Vehicle Slope	0.024	0.08	630.00	0.30	.766

In phase 2, none of the treatment conditions had a significant impact on the body weights. When phase day number was included in the analysis, there was also no pattern of significance on the body weights. In summation, it does not appear that the body weights of the mice were significantly influenced by the drug or day in phase.

Figure 12

Phase 2 Body Weights Multilevel Linear Regression Graph



Note. Other details as noted in Figure 5.

Table 9

Phase 2 (2%) Body Weights Multilevel Linear Regression Results

Multilevel Linear Regression Results					
Predictor	**Estimate**	**SE**	**df**	**t**	**p**
No Treatment	23.448	1.14	7.38	20.52	< .001
Vehicle	-0.283	0.46	444.00	-0.62	.535
1 mg/kg Dose	0.052	0.31	444.00	0.17	.867
Vehicle	0.040	0.99	444.01	0.04	.968
3 mg/kg Dose	0.317	0.53	444.01	0.60	.547
Vehicle	-0.069	1.70	444.01	-0.04	.968
6 mg/kg Dose	0.763	0.82	444.01	0.93	.352
Vehicle	4.659	3.19	444.01	1.46	.144
No Treatment	-1.870	1.84	444.01	-1.02	.310
No Treatment Slope	0.117	0.06	444.00	1.85	.066
Vehicle Slope	-0.064	0.08	444.00	-0.80	.426
1 mg/kg Slope	-0.087	0.06	444.00	-1.34	.181
Vehicle Slope	-0.077	0.07	444.00	-1.05	.295
3 mg/kg Slope	-0.096	0.06	444.00	-1.48	.141
Vehicle Slope	-0.078	0.07	444.00	-1.05	.293
6 mg/kg Slope	-0.100	0.07	444.00	-1.54	.124
Vehicle Slope	-0.172	0.08	444.00	-2.09	.038
No Treatment Slope	-0.063	0.07	444.00	-0.92	.359

Conclusion

The most pronounced effects of the olanzapine treatment appeared to be on the total amount of liquid consumed by the mice. As the drug dose increased, the mice consistently consumed less liquid from both bottles and consumption trended back towards baseline during each vehicle condition. There also seems to be an effect of the drug on food consumption in the mice. It is difficult to make any definitive conclusions about food consumption because of the food grinding behaviors that were present during the latter half of the two phases. It is possible that the drug promoted food grinding behaviors when the mice were being treated with higher doses because of the consistency in when the behaviors were performed, but further observation

and research would need to be done to gather more information. Based on the current data, it does seem as though food consumption fluctuated consistently with the drug dosing schedule.

Although treatment significantly decreased the amount of liquid being consumed, preference for sucrose over water increased during the drug treatment conditions. The vehicle condition following the initial control condition did deviate significantly from the baseline which indicates an effect of the vehicle by itself. This was the first time the mice were introduced to cookie dough during the experiment, so it is possible that this had an unanticipated effect on sucrose preference since this effect did not repeat in phase 2. Sucrose preference during the final vehicle condition was significantly greater than the baseline, but it is possible that the mice were experiencing withdrawal effects after being taken off of the highest and final dose of olanzapine. Further testing and observation would need to be done in order to make any definitive conclusions about withdrawal effects.

Although the current study did not find an impact of the drug on body weights, current literature suggests that weight gain is a recurring issue in patients prescribed olanzapine. The data from this experiment do not support the clinical implication that sub-chronic dosing of olanzapine induces a preference for high sugar diets but given that weight gain was not observed it remains possible that when weight gain does occur, it is associated with increased preference for obesogenic foods. It is possible that diet preference could be an underlying mechanism contributing to the weight gain in question. Since food grinding is not a behavior that humans engage in (specifically, spitting out the food after chewing on it), it is possible that clinical populations are engaging in altered eating or snacking patterns which could be an additional explanation for the weight gain. The decline in total liquid consumption suggests that olanzapine does have an effect on certain consumption-related behaviors. Since food and liquid intake both

appeared to be affected, it is possible that clinical populations are experiencing a shift in feelings relating to hunger and thirst.

A potential future direction of this project would be to add a third phase with a sucrose concentration in between 0.25% and 2%. The 0.25% concentration served as an effective starting point to observe effects at a low concentration, but the 2% sucrose concentration induced an exclusive preference which made it difficult to observe any increases in preference, but it did allow for the possibility of observing decreases. The current data suggest that a concentration of 1% could potentially allow for room to observe a change in preference without creating an initial exclusive preference.

Another modification that should be considered is increasing the length of time in between doses. In hindsight, it does not appear that four days was enough time in between doses to allow observation of any significant behavior changes. Also, because of the data gathered on total food consumption, it does appear that the vehicle might have affected thirst levels. In the future, a "No Treatment" phase could be implemented in between doses alongside a vehicle condition, or a different vehicle could be used.

In summary, further observation and investigation should be done to gather more information on the thirst and food-grinding related effects of olanzapine. Since it is difficult to get accurate data on food consumption when the mice are engaging in food-grinding, the current study was not able to draw any definitive conclusions on the effects of olanzapine on food consumption. For body weights, it is possible that there was an overall significant change from the start of phase 1 to the end of phase 2, but since analyses were only done within each phase and not between the two phases, there were also no strong results supporting an increase in body weights. Repeating the study with a different vehicle or with added "No Treatment" conditions

between the doses would potentially produce more clear results with liquid consumption and sucrose preference behaviors. Since it appears that there could be an effect of the dough itself on consumption and preference, repeating the experiment with the aforementioned modifications would allow for stronger distinctions to be made between vehicle and drug effects.

References

Choi, S., DiSilvio, B., Unangst, J., & Fernstrom, J. D. (2007). Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. Life Sci, 81(12), 1024-1030. doi:10.1016/j.lfs.2007.08.009

Christian, R., Saavedra, L., Gaynes, B. N., Sheitman, B., Wines, R., Jonas, D. E., Viswanathan, M., Ellis, A. R., Woodell, C., & Carey, T. S. (2012). Future research needs for first- and second-generation antipsychotics for children and young adults. Agency for Healthcare Research and Quality (US).

Cuerda, C., Velasco, C., Merchan-Naranjo, J., Garcia-Peris, P., & Arango, C. (2014). The effects of second-generation antipsychotics on food intake, resting energy expenditure and physical activity. Eur J Clin Nutr, 68(2), 146-152. doi:10.1038/ejcn.2013.253

Davoodi, N., Kalinichev, M., Korneev, S. A., & Clifton, P. G. (2009). Hyperphagia and increased meal size are responsible for weight gain in rats treated sub-chronically with olanzapine. Psychopharmacology (Berl), 203(4), 693-702. doi:10.1007/s00213-008-1415-1

Eagle, A. L., Mazei-Robison, M. and Robison, A. J. (2016). Sucrose preference test to measure stress-induced anhedonia. Bio-Protocol, 6(11). doi:10.21769

Elman, I., Borsook, D., & Lukas, S. E. (2006). Food intake and reward mechanisms in patients with schizophrenia: implications for metabolic disturbances and treatment with second-generation antipsychotic agents. Neuropsychopharmacology, 31(10), 2091-2120. doi:10.1038/sj.npp.1301051

Gothelf, D., Falk, B., Singer, P., Kairi, M., Phillip, M., Zigel, L., Poraz, I., Frishman, S., Constantini, N., Zalsman, G., Weizman, A., & Apter, A. (2002). Weight gain associated with increased food intake and low habitual activity levels in male adolescent schizophrenic inpatients treated with olanzapine. The American journal of psychiatry, 159(6), 1055–1057. https://doi.org/10.1176/appi.ajp.159.6.1055

Kuznetsova A, Brockhoff PB, Christensen RHB (2017). lmerTest Package: Tests in linear mixed effects models. Journal of Statistical Software, *82*(13), 1-26. doi: 10.18637/jss.v082.i13 (URL: https://doi.org/10.18637/jss.v082.i13).

Murashita, M., Kusumi, I., Inoue, T., Takahashi, Y., Hosoda, H., Kangawa, K., & Koyama, T. (2005). Olanzapine increases plasma ghrelin level in patients with schizophrenia. Psychoneuroendocrinology, 30(1), 106-110. doi:10.1016/j.psyneuen.2004.05.008

Muscat, R., & Willner, P. (1989). Effects of dopamine receptor antagonists on sucrose consumption and preference. Psychopharmacology, 99(1), 98–102. https://doi.org/10.1007/BF00634461

R Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Smith, G. C., Vickers, M. H., & Shepherd, P. R. (2011). Olanzapine effects on body composition, food preference, glucose metabolism and insulin sensitivity in the rat. Arch Physiol Biochem, 117(4), 241-249. doi:10.3109/13813455.2011.576681

Tarsy, D., Baldessarini, R. J., & Tarazi, F. I. (2002). Effects of newer antipsychotics on extrapyramidal function. CNS Drugs, 16(1), 23-45. doi:10.2165/00023210-200216010-00003

van der Zwaal, E. M., Janhunen, S. K., Luijendijk, M. C., Baclesanu, R., Vanderschuren, L. J., Adan, R. A., & La Fleur, S. E. (2012). Olanzapine and sibutramine have opposing effects on the motivation for palatable food. Behavioural pharmacology, 23(2), 198–204. https://doi.org/10.1097/FBP.0b013e3283512ca1

Wirshing D. A. (2004). Schizophrenia and obesity: impact of antipsychotic medications. The Journal of clinical psychiatry, 65 Suppl 18, 13–26.