Deletion of Mu Opioid Receptors Reduces Palatable Solution Intake in a Mouse Model of Binge Eating

MOP KO reduces palatable intake in mice

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Abstract

Binge eating in humans is driven by hedonic properties of food, suggesting that brain reward systems may contribute to this behaviour. We examined the role of mu opioid receptors (MOP) in binge eating by examining sweet solution intake in mice with genetic deletion of the MOP. Wildtype (WT) and MOP knockout (KO) mice had 4 hr access to food in the home cage combined with limited (4 hr) access to sucrose (17.1% w/v) or saccharin (.09% w/v), or continuous (24 hr) access to sucrose. Only limited access groups exhibited binge intake, measured as increased solution consumption during the first hour. KO mice consumed less solution and food during the first hour as well as less food each day compared to WT mice. Limited access groups consumed more food and gained more weight than continuous access groups, and the effect was magnified in saccharin-consuming mice. Indeed, the increased food consumption in animals given limited access to saccharin was so excessive that caloric intake of this group was significantly higher than either of the sucrose groups (limited or continuous access). Within this group, females consumed more food per bodyweight than males, highlighting important sex differences in feeding behaviours under restricted access schedules.

Keywords: BED; Mu opioid receptor; Saccharin; motivation; reward; feeding

Introduction

Binge eating disorder (BED), the most prevalent of all eating disorders (Kessler et al., 2013; Solmi et al., 2016), is associated with detrimental health outcomes including obesity (Grucza et al., 2007; Stojek and MacKillop, 2017) and several mental health conditions (Javaras et al., 2008; Solmi et al., 2016). Treatments for BED are not widely available and generally ineffective, partly because the etiology of the disorder is poorly understood (Hutson et al., 2017). Emerging evidence points to alterations in brain reward circuits in BED patients (Balodis et al., 2015; Kessler et al., 2016), matching reports of increased dopamine (DA) release in the nucleus accumbens (NAcc) of rats following sucrose bingeing (Rada et al., 2005). In the context of motivated behaviours, disruptions in mesolimbic DA function would impact binge eating by altering 'wanting' of food rewards (Kelley and Berridge, 2002).

In contrast, the 'liking' component of natural rewards is mediated through opioid systems, specifically the mu opioid receptor (MOP) (Castro and Berridge, 2014). For instance, intra-NAcc injections of MOP agonists produce hyperphagia of highly palatable food (Katsuura and Taha, 2014; Nogueiras et al., 2012; Zhang and Kelley, 2002); opioid and selective MOP antagonists have the opposite effect (Katsuura and Taha, 2014; Sahr et al., 2008; Ward et al., 2006). MOPs appear to be linked, specifically, to hedonic responses associated with preferred foods (Giuliano et al., 2012; Nogueiras et al., 2012; Ostlund et al., 2013), which may explain why blockade of MOPs reduces subjective ratings and *ad libitum* intake of preferred foods in BED patients (Drewnowski et al., 1995; Ziauddeen et al., 2013). Together, clinical and preclinical data suggest that MOPs may contribute to binge eating by regulating the hedonic aspects of palatable food.

We tested the role of MOPs in binge eating by assessing intake of highly palatable food (sucrose or saccharin) and regular chow in mice lacking MOPs. Binge eating, defined as excessive consumption within a discrete period of time, was induced using a limited access protocol (Corwin et al., 2011) adapted for mice (Yasoshima and Shimura, 2015). We focused, exclusively on sweet solutions, rather than sweet/fat combinations, as the two commodities induce distinct neural and behavioural adaptations (Avena, 2010).

Materials and Methods

Subjects

Twelve male and twelve female mice lacking MOP receptors and their wildtype (WT) controls (WT female n=30; male n=24) were single housed under standard light, temperature, and humidity conditions (12 hr light-dark cycle, 22 ± 2 °C, 55 ± 10% humidity). Knockout (KO) mice were generated by homologous recombination (Matthes et al., 1996) on a genetic background of 50% C57/BL6J:50% 129svPas. Mice weighed 23-46.7g at the start of the experiment, ranging in age from four months to approximately one year (WT: 17.7-32.8 weeks; MOP KO: 18.5-58.8 weeks).

Research was conducted in accordance with the European Communities Council Directive of 22 September 2010 (directive 2010/63/UE). Experiments were approved by the local ethical committee (Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg CREMEAS) and findings are reported following the ARRIVE Guidelines for experiments involving animals.

Sucrose Consumption

The protocol for sucrose consumption, including solution concentrations and access periods, was based on a procedure that induces sucrose bingeing in mice (Yasoshima and

Shimura, 2015). Briefly, MOP KO and WT mice were randomly assigned to one of three access conditions: limited sucrose (4 hr sucrose and food, 4SUC/4F); limited saccharin (4 hr saccharin and food, 4SAC/4F); or continuous sucrose (24 hr sucrose and 4 hr food, 24SUC/4F). As far as possible, sex, age, and initial weight were counterbalanced across groups. Sucrose and saccharin were presented at concentrations that are equally preferred in mice (17.1% and .09% w/v, respectively).

Mice were habituated to single housing and water presentation in two sipper tubes in the home cage for a minimum of six days. Over the next 14 days, beginning 2 hr into the light cycle, mice were presented with standard chow and solution according to their group assignment. Water was available *ad libitum*. Solution (mL) and chow (g) intake were measured 1 hr, 4 hr, and 24 hr following presentation. Binge intake was assessed as significantly higher solution consumption during the first hour of access. Animals were weighed daily and sacrificed at the end of the intermittent access period.

Statistical Analyses

Solution and food intake were analyzed using a Linear Mixed Model (LMM) analysis (Winter, 2013) in which consumption (solution, food, kilocalories) was assessed as a function of group, day, sex, and weight. LMM analyses accounts for both fixed and random effects, the latter reflecting individual differences in baseline intake. Degrees of freedom were calculated using the Welch-Satterthwaite equation (pooled degrees of freedom) as there is no assumption that underlying population variances are equal (Satterthwaite, 1946). Group differences across sessions were analyzed using a likelihood ratio test (LRT) (Luke, 2017) that compares goodness of fit of two models: the full model against one that combines two groups of interest into a single group. Statistically significant effects indicate that the two groups are distinct.

Results

Figure 1 shows that limited access to a sweet solution induces binge intake in both WT and MOP KO mice, confirmed by a significant escalation of sucrose intake across 14 days for the 4SUC/4F WT ($t_{(1,424)}$=7.906; P<.001), 4SUC/4F MOP KO ($t_{(1,399)}$=4.545; P<.001), 4SAC/4F WT ($t_{(1,412)}$=3.878; P<.001), and 4SAC/4F MOP KO ($t_{(1,403)}$=2.890; P=.003) groups (Figure 1A). Intake during the first hour of access did not increase across sessions in WT or KO mice given continuous access to sucrose (i.e., 24SUC/4F groups). LRT analysis revealed that the rate of increased solution intake during the first hour was higher in WT compared to MOP KO mice given limited access to sucrose (X^2(2)=12.174; P=.002) and saccharin (X^2(2)=8.8796; P=.01).

Chow consumption during the first hour also increased across sessions in all groups [4SUC/4F MOP KO ($t_{(1,622)}$=2.688; P=.007); 4SAC/4F WT ($t_{(1,639)}$=4.983; P<.001) and MOP KO ($t_{(1,625)}$=5.238; P<.001); 24SUC/4F WT ($t_{(1,622)}$=2.501; P=.012) and MOP KO ($t_{(1,624)}$=3.284; P<.001)], with the exception of 4SUC/4F WT mice ($t_{(1,644)}$=1.100; P=.244). The rate of increased chow intake during the first hour was significantly different in WT and MOP KO mice in the 4SUC/4F group (X^2(2)=11.291; P=.004), but not in the other two groups: 4SAC/4F (X^2(2)=1.342; P= .511) and 24SUC/4F (X^2(2)=5.935; P=.051) (Figure 1B).

In order to assess overall differences in consumption, we conducted an analysis of variance (ANOVA) on all measures, using access group and genotype as between subjects' factors. This yielded a generally consistent pattern of results with no significant group X genotype interaction for any measure of intake or body weight (Ps>.05), although WT mice drank more water than MOP KO mice ($F_{(1,61)}$=5.686; P=.020) (Figure 1C).

Figure 1 D shows that daily sweet solution intake did not differ between WT and MOP KO groups [4SUC/4F ($X^2(2)=2.437$, df=2; $P=.296$); 4SAC/4F ($X^2(2)=5.775$; $P=.056$); and 24SUC/4F ($X^2(2)=1.1306$; $P=.568$)]. LMM analysis also revealed significant escalation of daily solution intake across sessions, only in limited access groups [4SUC/4F WT ($t_{(1,238)}=9.877$; $P<.001$) and MOP KO ($t_{(1,224)}=6.479$; $P<.001$); 4SAC/4F WT ($t_{(1,228)}=9.990$; $P<.001$) and MOP KO ($t_{(1,227)}=7.092$; $P<.001$)].

All six groups showed significant increases in daily chow consumption across sessions, verified by LMM analysis in WT [4SUC/4F ($t_{(1,601)}=3.699$; $P<.001$); 4SAC/4F ($t_{(1,591)}=9.418$; $P<.001$); 24SUC/4F ($t_{(1,574)}=3.450$; $P<.001$)] and MOP KO [4SUC/4F ($t_{(1,574)}=4,071$; $P<.001$) ; 4SAC/4F ($t_{(1,578)}=9.909$; $P<.001$) ; 24SUC/4F ($t_{(1,572)}=4.130$; $P<.001$)] groups. The rate of daily chow intake was higher in WT groups with limited access to sucrose ($X^2(2)=10.112$; $P=.006$) and saccharin ($X^2(2)=6.492$; $P=.039$). (Figure 1E). In addition, WT mice gained more weight than MOP KO mice ($F_{(1,62)}=6.927$; $P=.010$), with the two limited access groups (4SUC/4F, 4SAC/4F) gaining more than the continuous access (24SUC/4F) group ($F_{(2,62)}=7.374$; $P<.001$) (Figure 1F).

Data presented in Figure 2 show that MOP KO mice consumed less sweet solution ($F_{(1,62)}=12.090$; $P<.001$) and food ($F_{(1,62)}=9.594$; $P=.002$) than WT mice during the first hour of access (Figures 2A and 2B), as well as less total food ($F_{(1,54)}=11.449$; $P<.001$) across sessions (Figure 2E). Total daily intake of sweet solution did not differ across genotypes ($F_{(1,62)}=.19$; $P=.665$) (Figure 2D). In addition, solution intake during the first hour was significantly higher in both limited access groups ($F_{(2,62)}= 28.217$; $P<.001$), whereas food intake was increased only in the 4SAC/4F group ($F_{(2,62)}= 24.384$; $P<.001$). Total daily intake followed a similar pattern with the continuous access group (24SUC/4F) consuming more sweet solution than the limited access

groups ($F_{(2,62)}$=49.381; P<.001), and the 4SAC/4F group consuming more food ($F_{(2,54)}$=47.367; P<.001) and water ($F_{(2,61)}$=10.329; P<.001) than the other two groups.

Increased food intake in the saccharin group could simply reflect compensation for the lack of calories in artificial sweetener. To assess this, we compared total calorie intake across groups using the following calculations: chow = 3.952 Kcal/g; sucrose solution= 0.114 Kcal/mL; saccharin=0.00366 Kcal/mL. Analysis of these data yielded a significant group effect, with the saccharin group consuming more calories than either sucrose group in the first hour ($F_{(2,54)}$= 13.124; P<.001) (Figure 2C) and across the entire session ($F_{(2,54)}$= 29.324; P<.001) (Figure 2F). Again, WT mice consumed more calories than MOP KO mice ($F_{(1,54)}$= 10.121; P=.002) (Figures 2C and 2F).

We also examined sex differences in all intake measures (data not shown) using LMM analysis, revealing that WT females in the 4SAC/4F group consumed more solution (X^2(2)=10.170; P=.006) and chow (X^2(2)=13.753; P=.001) than males in the first hour, as well as more chow across the session (X^2(2)=33.666; P<.001). There were no sex differences in any intake measure of WT animals given access to sucrose (i.e., 4SUC/4F or 24SUC/4F). ANOVA on total consumption of both genotypes across sessions confirmed that females in the 4SAC/4F group consumed more chow than males in the first hr ($F_{(1)}$=9.343; P=.006), and over the 4-hr access period ($F_{(1)}$=33.333; P<.001). In the latter analysis, both the genotype ($F_{(1)}$=7.160; P=.015) and sex X genotype interaction ($F_{(1)}$=4.685; P=.044) were statistically significant.

Discussion

We successfully reproduced a model of binge intake in mice (Yasoshima and Shimura, 2015), manifested as excessive consumption of a sweet solution within the first hour of access. Mice given limited access to food, but not sucrose (i.e., 24SUC/4F group), did not display binge

intake, confirming that restriction of a palatable substance contributes to this maladaptive behaviour. In our preliminary experiments (data not shown), mice given limited access to sucrose and unlimited access to food (i.e., 4SUC/24F) also displayed binge intake, although it was reduced compared to the group with restricted access to both commodities (4SUC/4F). Food restriction, therefore, may exacerbate hyperphagic responses to palatable food. This could occur through physiological stress responses which, themselves, induce binge eating in mice (Micioni Di Bonaventura et al., 2014). Human studies support a relationship between stress and binge eating in that chronically high levels of glucocorticoids may trigger intake of 'comfort' foods (Dallman et al., 2003) and stressful life events initiate both binge eating and cortisol production in BED patients (Gluck, 2006).

Our study also confirmed reduced consumption of food and sweet solution in mice lacking MOPs (Ostlund et al., 2013). The effect was particularly apparent in our measure of binge intake (1^{st} hr solution consumption), fitting evidence that MOPs play a role in hedonic responses of binge eating in humans (Cambridge et al., 2013). This genotype profile is also revealed in conditions of food deprivation (Ostlund et al., 2013) or increased effort to obtain a reward (Papaleo et al., 2007; Roberts et al., 2000). In contrast, MOP KO and WT mice show no differences in food intake or seeking responses when access to palatable food is increased or continuous (Papaleo et al., 2007; Tabarin et al., 2005). These differences could reflect a critical role of MOPs in stress responses (Ide et al., 2010; Labuda et al., 2000), which would be increased under food restriction. A MOP contribution to binge eating may occur through interactions with orexigenic neurons (Castro and Berridge, 2017), which amplify hedonic or liking responses to a sweet solution (Castro and Berridge, 2014). This is supported by evidence that BED patients exhibit a loss of MOP availability in the NAcc (Majuri et al., 2017), an area

described as a 'hedonic hotspot' in food reward (Castro et al., 2015). Our findings are in general agreement with studies showing decreased consumption of palatable food with MOP antagonism (Giuliano and Cottone, 2015; Taha, 2010), although this effect may not involve the NAcc (Lardeux et al., 2015). We also observed that MOP KO mice gained less weight than their WT controls, which could reflect altered physiological and metabolic responses to food (Wen et al., 2009) as well as increased energy homeostasis and disrupted hunger cues (Tabarin et al., 2005). Indeed, MOPs may have a marginal role in satiation processes in that anticipatory or 'wanting' response for food are not affected by food deprivation in MOP KO mice (Kas et al., 2004). Finally, we cannot rule out the possibility that alterations in functional brain connectivity, characteristic of MOP KO mice (Mechling et al., 2016), contribute to altered behavioural responses in this genotype.

Somewhat surprisingly, mice given limited access to saccharin ate more food than mice with either limited or continuous sucrose access, even beyond an expected compensation for caloric differences in natural and artificial sweeteners. The increased food intake in saccharin bingeing animals was not dependent on the presence or absence of MOPs, but may be linked to sex differences: binge intake of saccharin escalated more quickly across sessions in females than males, whereas all other intake measure were similar in the two sexes. This matches previous findings that female, but not male, rats exhibit an increased preference for noncaloric, but not caloric, sweeteners (Valenstein et al., 1967). These findings emphasize the need to critically evaluate the continued and widespread use of artificial sweeteners, particularly as abstinence from saccharin (Aoyama et al., 2014), like sucrose (Grimm et al., 2013), elicits craving in rats and non-nutritive sweeteners induce physiological changes mimicking those of nutritive compounds (Tucker and Tan, 2017). Furthermore, although we did not observe this effect in our

mice, non-caloric sweeteners can induce higher levels of weight gain than natural sugars (Feijó et al., 2013), possibly through an increase in orexigenic peptides (Furudono et al., 2006; Gaysinskaya et al., 2011).

In sum, rodent models of sucrose bingeing provide valuable insight into the behavioural and biological underpinnings of maladaptive eating. Restricting access to palatable food increases the propensity to binge, leading to alterations in reward processing (Smail-Crevier et al., 2018) as well as neurophysiological changes associated with compulsive responding (Maracle et al., 2018). We now identify a contribution of MORs to binge eating, findings that may translate to clinical treatment (Ziauddeen et al., 2013). Our study also points to potential pitfalls in substituting artificial for natural sweeteners, and highlights the need to understand gender differences in eating behaviours.

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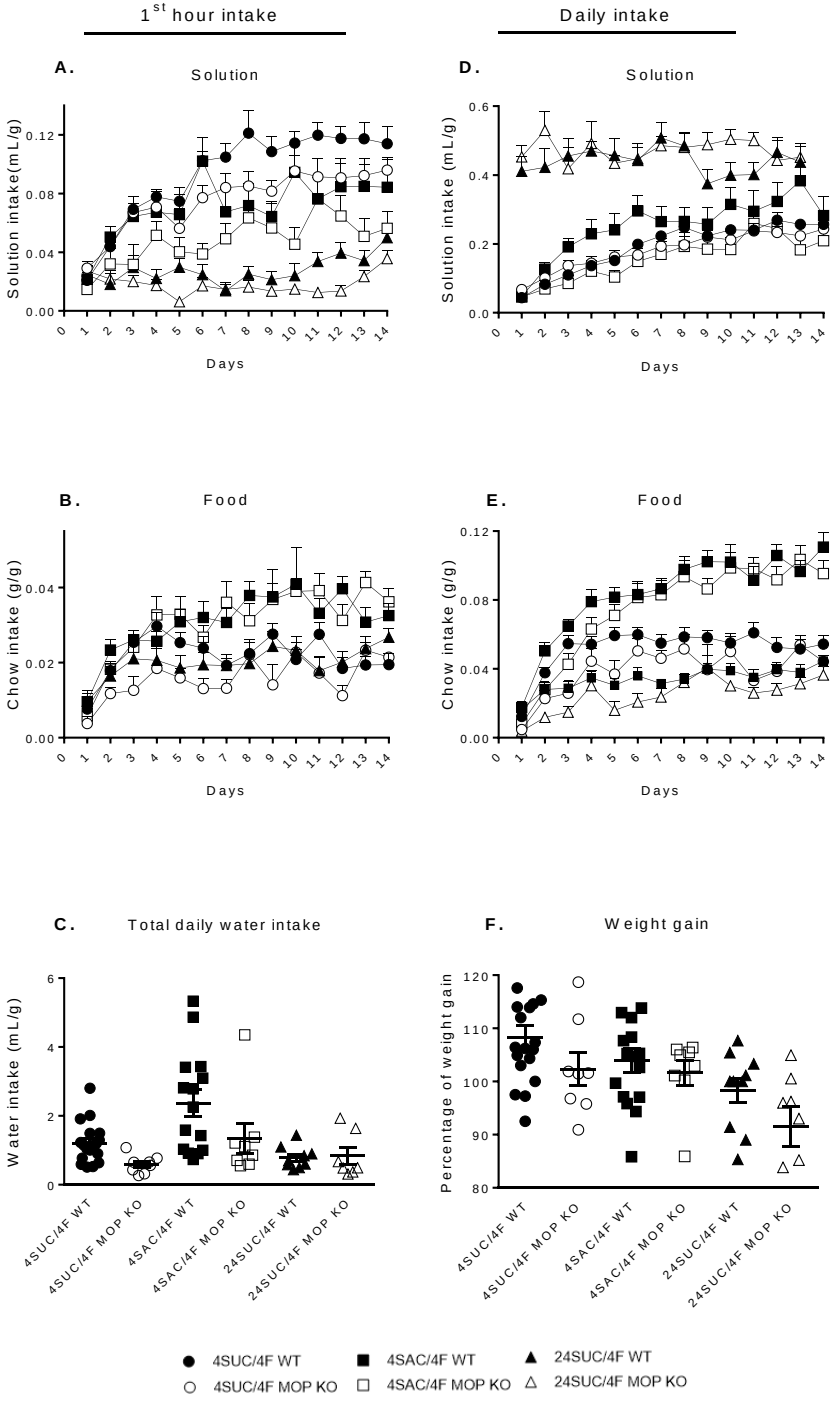
Figure Captions

Figure 1. Limited access to sweet solution produces binge intake

Mice were given daily access to sucrose, saccharin, and food for 14 days as follows: 4SUC/4F=4 hr access to sucrose and food; 4SAC/4F=4 hr access to saccharin and food; 24SUC/4F=24hr access to sucrose and 4 hr access to food. Data are presented as mean intake per body weight (mL/g and g/g) of solution (A,D) or food (B,E) during the first hour of access (A,B), and over each 24-hr period (D,E). Total water intake (C) and percentage weight gain from baseline (F) were calculated across the 14 days. Error bars represent standard error of the mean. MOP KO=mu knockout; WT=wildtype

Figure 2. Limited access to saccharin increases total calories consumed

Mice were given daily access to sucrose, saccharin, and food for 14 days as follows: 4SUC/4F=4 hr access to sucrose and food; 4SAC/4F=4 hr access to saccharin and food; 24SUC/4F=24hr access to sucrose and 4 hr access to food. Data are presented as mean total solution (A,D) or food (B,E) intake per body weight (mL/g and g/g), summed across 14 sessions. Intake was measured during the first hour of access (A,B) and over each 24-hr period (D,E). Caloric intake per body weight was summed across 14 sessions for the 1st hour of intake (C) and for each 24-hr period (F). Error bars represent standard error of the mean. MOP KO=mu knockout; WT=wildtype


1st hour intake
Daily intake
A.
Solution
Solution intake(mL/g)
Days
D.
Solution
Solution intake (mL/g)
Days
B.
Food
Chow intake (g/g)
Days
E.
Food
Chow intake (g/g)
Days
C.
Total daily water intake
Water intake (mL/g)
4SUC/4F WT
4SUC/4F MOP KO
4SAC/4F WT
4SAC/4F MOP KO
24SUC/4F WT
24SUC/4F MOP KO
F.
Weight gain
Percentage of weight gain
4SUC/4F WT
4SUC/4F MOP KO
4SAC/4F WT
4SAC/4F MOP KO
24SUC/4F WT
24SUC/4F MOP KO
4SUC/4F WT
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4SAC/4F WT
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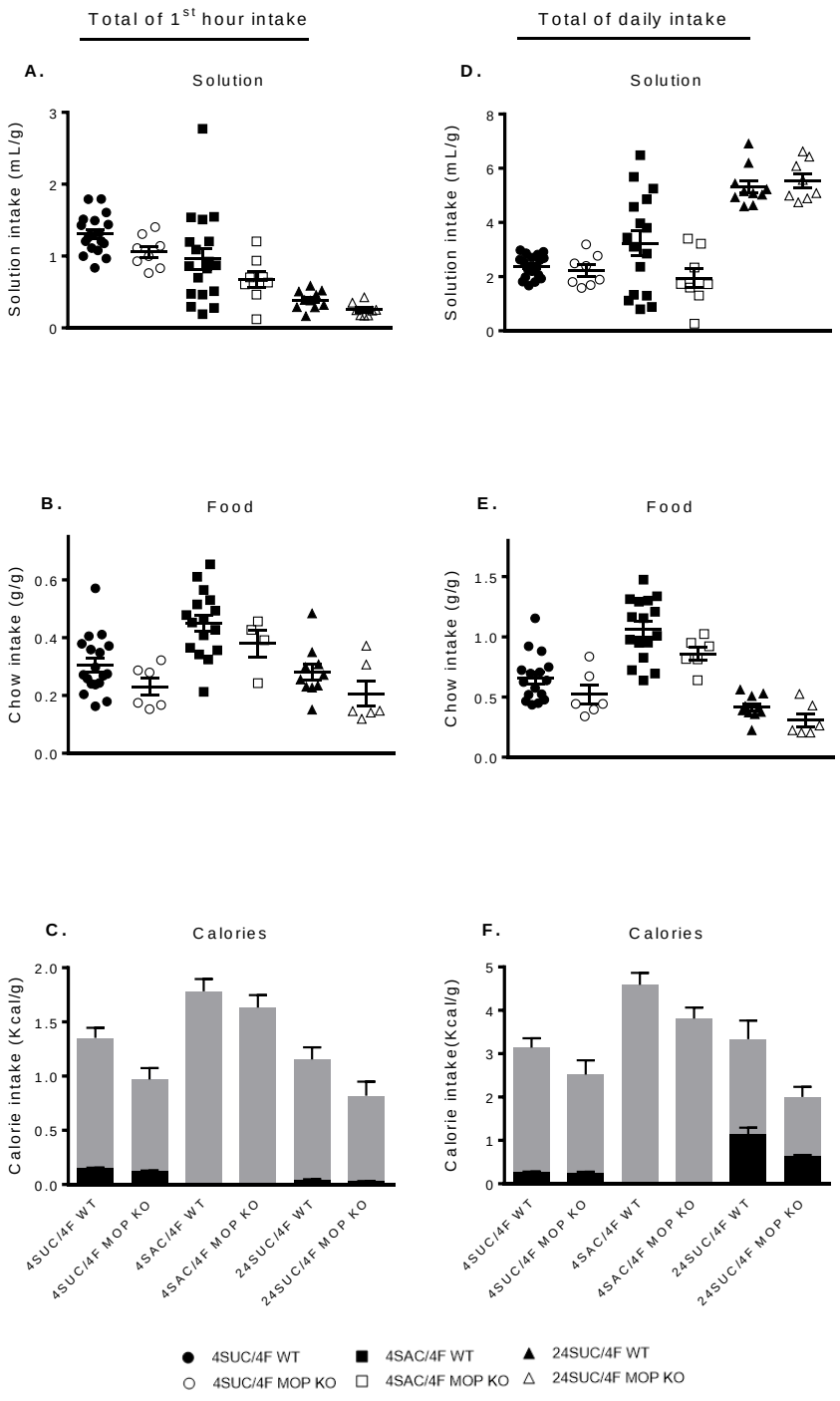
24SUC/4F WT
24SUC/4F MOP KO


Total of 1st hour intake
Total of daily intake
A.
Solution
Solution intake (mL/g)
D.
Solution
Solution intake (mL/g)
B.
Food
Chow intake (g/g)
E.
Food
Chow intake (g/g)
C.
Calories
Calorie intake (Kcal/g)
F.
Calories
Calorie intake(Kcal/g)
4SUC/4F WT
4SUC/4F MOP KO
4SAC/4F WT
4SAC/4F MOP KO
24SUC/4F WT
24SUC/4F MOP KO