Title: A simple action reduces food intake and obesity in mice

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Abstract:

Increasing dietary fat causes over-eating and weight gain in multiple animal species, suggesting that increases in dietary fat are sufficient to cause obesity. However, in such experiments high-fat diet is typically provided freely to animals, so it remains unclear how increasing dietary fat would impact body weight if the high-fat diet required more effort to obtain. Here, we hypothesized that unrestricted access is necessary for mice to overeat high-fat diets, and the corollary that requiring mice to perform small amounts of work to obtain high-fat diets would reduce food intake and weight gain in a high-fat diet model of obesity. To test this hypothesis, we utilized home-cage based feeding devices that either provided food freely or required mice to briefly poke their noses into a port – a simple action that is easy for them to do - to receive food. With both low-fat and high-fat diets, requiring mice to nose-poke just one time to access food strongly reduced food intake, and in the case of high-fat diet prevented the development of obesity. We conclude that unrestricted access to food promotes overeating in mice, and that making mice perform a simple action such as a nose-poke to access food reduces over-eating and weight gain. We posit that ubiquitous access to low-cost food is a critical contributor to the human obesity epidemic, and that changes in the availability of unhealthy food may be part of the solution for addressing the obesity epidemic.

Introduction

The United States Department of Agriculture (USDA) tracks historical trends in food availability (1), revealing that total available calories per capita in the US rose by about 23% between 1970 and 2014, accounted for primarily by changes in available grains and fats (2,3). While the USDA data does not quantify food consumption over this period, an orthogonal dataset utilizing dietary recall, the National Health and Nutrition Examination Survey (NHANES), reported a 12-15% increase in calorie consumption between 1970 to 2010 (4). While increases in food consumption are likely driving the obesity epidemic (5,6), questions still remain: Why are Americans eating more than they were eating in 1970? Has something changed in our food supply or environment that is causing us to overeat? Possible explanations include ubiquitous access to unhealthy foods (7,8), changes in food formulations and processing (5), interactions between genetic predispositions and the modern food supply (9), chemicals in the environment including those that disrupt endocrine function (10,11), disruptions in sleep and circadian rhythms due to changes in the modern lifestyle (12), and other causes (9,13). As it is challenging to isolate specific factors in humans, laboratory animals have often been used to test how specific factors alter body weight.

A large body of literature suggests that changing the nutritional profile of an animal's diet can modify total calorie intake and body weight. Specifically, increasing the fat content of an animals diet induces over-eating and weight gain in multiple species, including monkeys, dogs, pigs, hamsters, squirrels, rats, and mice (14,15). High-fat diets have been used in this way for more than 75 years to model obesity in animals (15,16). The validity of the "high-fat diet" model of obesity relies on observational studies showing that higher dietary fat consumption is associated with higher obesity prevalence in humans (17,18), as well as interventional studies demonstrating that low-fat diets produce modest, but significant, weight loss in humans (19,20). However, in animal models of high-fat diet induced obesity, high-fat diets are typically placed in a food hopper in the animal's cage, providing unrestricted access at all times. While this may

replicate certain aspects of how ubiquitous high-fat foods have become in modern life, it fails to test whether such unrestricted access is a critical component of high-fat diet induced obesity in animals. We hypothesized that unrestricted access is necessary for animals to overeat high-fat diets, and the corollary that requiring mice to perform small amounts of work to obtain food would reduce intake and weight gain in a high-fat diet model of obesity.

To test these hypotheses, we designed feeding devices that allowed us to measure food intake over multiple days or weeks in the rodent home-cages. We first used the Feeding Experimentation Device version 3 (FED3) (21) to examine intake and meal patterns for grain-based food pellets. A strength of using FED3 in this manner was that we were able to deliver food as pre-formed 20mg food pellets, allowing for rich analysis of patterns of intake. This allowed us to test how many pellets mice took when the pellets were provided freely, or when mice had to perform a small amount of work (one nose-poke) to receive each pellet. In a multi-site study that included labs from 3 countries, we observed that requiring mice to nose-poke to receive a pellet cause a strong (>30%) reduction in how many pellets they took, supporting our primary hypothesis. We next performed an analogous set of experiments using high-fat diet, again finding that requiring mice to perform just one single nose-poke reduced how much high-fat diet mice took, and strongly attenuated high-fat diet induced weight gain. Our experiments support our hypothesis that a small action – one nose-poke – can reduce food intake and diet-induced weight gain, and that unrestricted access to unhealthy foods may be a critical contributor to the human obesity epidemic.

Results

Mice take less food when they have to nose-poke to receive it

We first tested the hypothesis that mice would take less food if they had to perform an action to obtain it, compared to when that same food was freely available. We tested this with the Feeding Experimentation Device version 3 (FED3), a smart pellet dispensing device that can dispense food pellets and log every pellet delivery over multiple days in the home-cage (21). The FED3 was set in either a free-feeding mode (Free) or a fixed-ratio 1 (FR1) mode. In the Free mode, 20mg grain-based food pellets (Bio-serv F0163) were always available in the pellet well, and pellets were replaced automatically whenever a mouse removed one. In the FR1 mode, the mouse had to break a photo-beam sensor with its nose (termed: a nose-poke) to cause FED3 to dispense each 20mg food pellet. There was no limit on how many pellets mice could obtain in either task and in both tasks FED3 was their only source of food. Free and FR1 data was collected from 105 mice (72M/33F) across 10 labs in three countries (Fig 1A). Mice completed at least one day of Free followed by at least one day of FR1, and the last day of Free and first

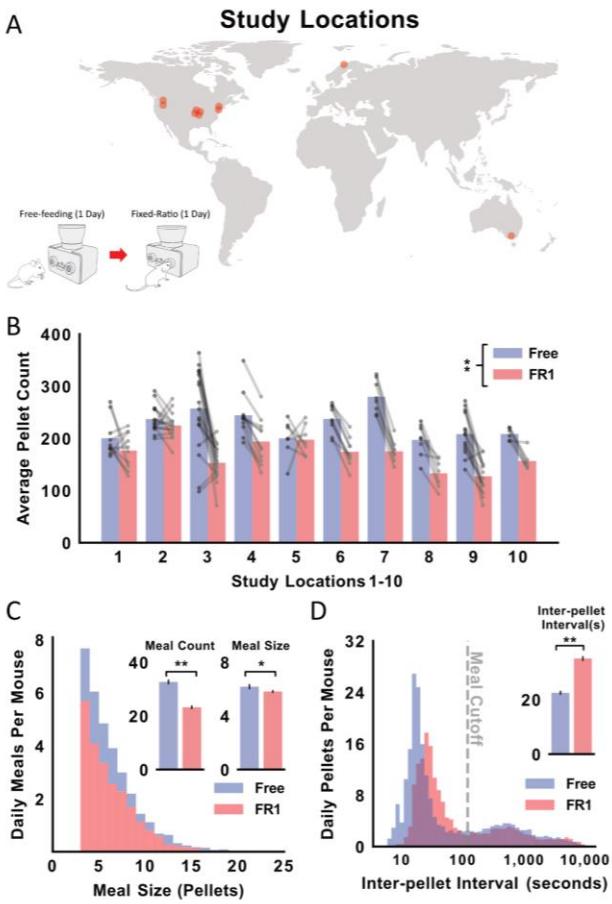

Figure 1: Mice take more food during free-access (Free) vs. fixed-ratio 1 (FR1) access. A. Locations of labs from which FED3 data was collected (n=10 study locations). **B**. Average pellet count for mice on the Free or FR1 task in ten study locations (n=105 mice). **C**. Histogram of meal sizes for Free vs FR1 tasks (n=105). (Insets) Average number and size of meals in each task. **D**. Histogram of inter-pellet intervals with a meal cutoff line. (Inset) Average inter-pellet interval for free vs FR1 task. ** denotes $p<0.001$ * denotes $p<0.05$.

day of FR1 were analyzed. Consistent with our hypothesis, mice took an average of 230 pellets (14.7Kcal) in the Free day and 170 pellets (10.9Kcal) in the FR1 day (significant effect of task: $F_{1,190} = 120.6$, $p<0.001$, Fig 1B), approximately a 35% reduction in pellets taken. This is consistent with a prior report using FED3 in these same tasks (22).

We next examined how mice altered their patterns of pellet retrieval on each task. Meals were defined based on the peaks in the inter-pellet interval distribution (Fig 1D), where pellets within 2 minutes of each other were considered the same meal, and breaks larger than 2 minutes initiated a new meal (21). Mice initiated ~39% fewer meals on FR1 vs. Free, which accounted for most of the difference in pellets taken (FR1: 23.2 meals/day, Free: 32.6 meals/day, paired t-test, $p < 0.001$, Fig 1C). The average meal size was also ~7% smaller during FR1 than Free (FR1: 5.8 pellets, Free: 6.2 pellets, paired t-test, $p = 0.04$), and mice took pellets more slowly within meals in FR1 vs Free (FR1: 35.0 seconds between pellets, Free: 22.5 seconds between pellets, paired t-test, $p < 0.001$, Fig 1D). Differences in pellets taken were not specific to one part of the circadian cycle (Fig S1A) and occurred in both male and female mice (paired t-tests, $p<0.005$ for both, Fig S1B). Together, our results show that mice took fewer food pellets when they were required to perform a single nose-poke to obtain them, vs. when the same pellets were freely available.

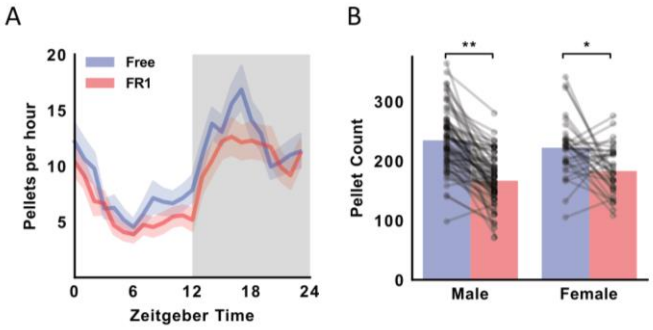

Figure S1: Differences in pellets taken on free-access than fixed-ratio 1 do not depend on circadian cycle or sex of mice. A. Circadian plot showing average pellets per hour over 24 hours for Free vs FR1 tasks. **B**. Pellet count for males (n=72) and females (n=33) in Free vs FR1 tasks. ** denotes p<0.001 * denotes p<0.05.

Why did mice take fewer pellets when a nose-poke was required?

We considered factors that might explain why mice took fewer pellets on FR1 vs. Free dispensing tasks. First, we considered if mice may have taken fewer pellets because they did not understand how to operate the FR1 task, and therefore could not efficiently obtain pellets from FED3. However, mice achieved over 80% accuracy (active over total nose-poke counts) within the first four hours of experiencing the FR1 task, confirming that they rapidly learned how to operate FED3 to obtain pellets (Fig 2A). We also trained a new group of 7 female mice for 5 days on the Free task followed by 5 days of the FR1 task, followed by two more 2-day blocks of Free and FR1 (Fig 2B). Mice took fewer pellets on each presentation of the FR1 task vs. the Free task (significant effect of Task: $F(1,6) = 7.7$, $p = 0.03$, no significant effect of Repetition or

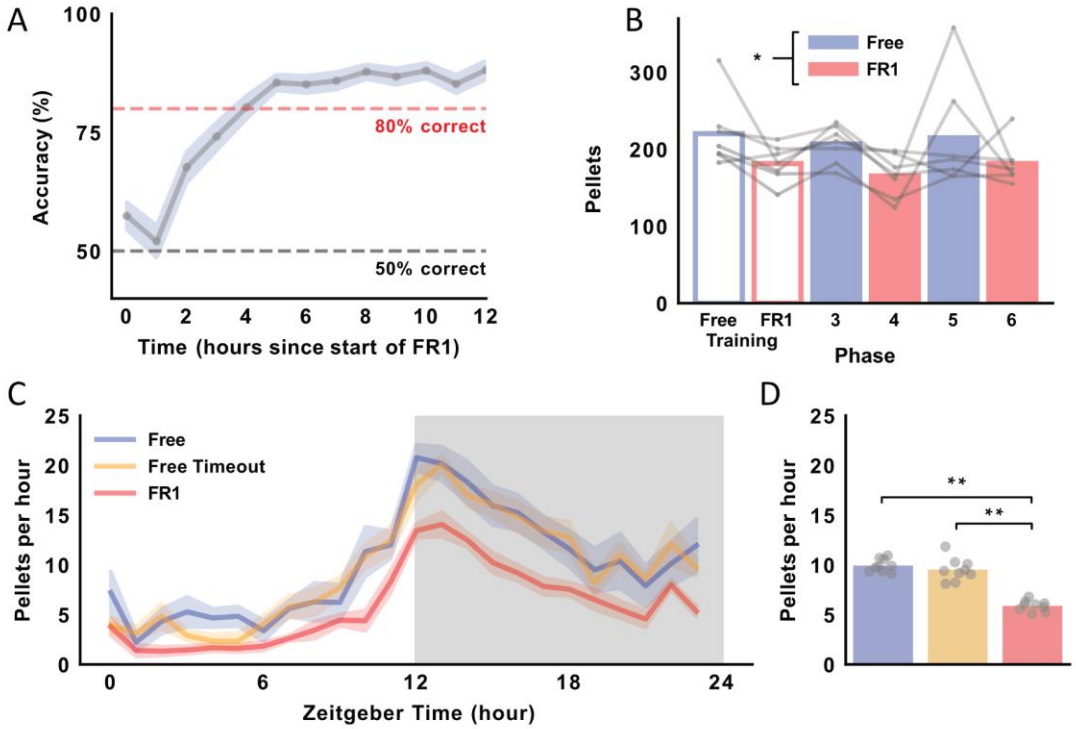

Figure 2: An exploration of factors that may control differences in pellets taken on Free vs. FR1 tasks. A. Poke accuracy from the first 12 hours of the FR1 task (n=105 mice). **B**. Number of pellets taken in alternating presentations of Free and FR1 tasks (n=7 mice). **C**. Average pellets taken over 24 hours during Free, FR1, and a Free timeout task where pellets were delayed by 30 seconds. **D**. Average pellets taken per hour over a 24-hour period in the Free, Free timeout and FR1 tasks. ** denotes $p<0.001$ * denotes $p<0.05$.

Task/Repetition interaction), confirming that repeated experience and presumably increased understanding of the FR1 task did not increase pellets taken in this task.

We also considered how mice consumed pellets more slowly within meals in the FR1 vs. Free task (Fig 1D), which would allow more time for satiety signals to reach the brain (23). To test if this accounted for the difference in total pellets taken, a new group of mice (n = 9, 4M/5F) completed a modified version of the Free task, where a 30s timeout (similar to the average time between pellets on the FR1 task, Fig 1D) was imposed before each new pellet was dispensed. This did slowed down their rate of eating (Fig S2A significant effect of Task $F(2,24) = 223.6$, $p < 0.001$) but did not decrease the overall number of pellets taken per day. These same mice also completed the FR1 task, which confirmed that requiring these mice to nose-poke decreased pellets taken (Fig 2C-D significant effect of Task $F(2,24) = 67.7$ $p < 0.001$, post-hoc t-tests Free vs. Free with Timeout: $p=0.52$ Free vs FR1 and Free-Timeout vs FR1, $p<0.001$ for both)

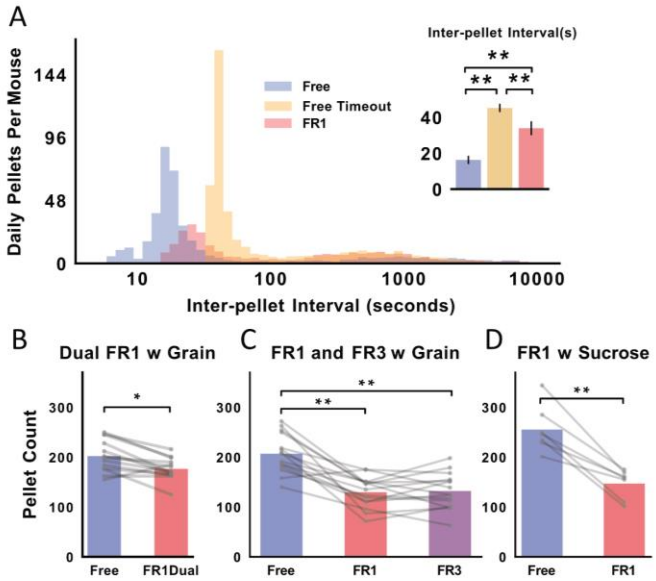

Figure S2: An exploration of factors that may control differences in pellets taken on Free vs. FR1 tasks. A. Histogram of inter-pellet intervals. (inset) Average inter-pellet interval for Free vs Free timeout vs FR1 task (n=9 mice) **B**. Pellets taken during Free feeding and an FR1 variation where pellets were delivered on either poke (n=13 mice). **C**. Pellets taken during Free feeding or a fixed-ratio 3 (FR3) task (n=15 mice). **D**. Pellets taken during Free feeding or an FR1 task with 100% sucrose pellets as the outcome (n=7 mice). . ** denotes p<0.001 * denotes p<0.05.

We next tested whether a simpler version of the FR1 task would also cause mice to take fewer pellets than the Free condition. Thirteen mice completed a variant of the FR1 task where both nose pokes caused the delivery of a pellet (termed: dual FR1). Mice still took significantly fewer pellets on this task than on Free (paired t-test, $p = 0.003$), suggesting that the difficulty of

the FR1 task was not responsible for reducing the number of pellets taken (Fig S2B). We next tested if increasing the task difficulty would cause even fewer pellets to be taken, by testing mice on Free, FR1 and a new fixed-ratio 3 (FR3) task. While there was a significant effect of Task on pellet count ($F(2, 27) = 22.971$, $p < 0.001$), there was no difference in pellet count between FR1 and FR3, which were both lower than Free (Fig S2C). Finally, we asked whether mice would reduce their pellet intake on FR1 if the outcome were sucrose. Mice still took significantly fewer sucrose pellets during the FR1 vs Free task, demonstrating the effect was present with both grain and sucrose pellets (paired t-test $p < 0.001$, Fig S2D). Overall, requiring mice to perform one nose-poke reduced pellets taken under multiple experimental conditions, and the reason for this reduction was not linked to differences in learning or timing of pellet delivery.

Do mice eat the excess pellets they take?

Despite taking fewer pellets in the FR1 task, it is not clear if mice *consumed* fewer pellets in the FR1 task than the Free task. To test this, we used indirect calorimetry to measure V_{O2}, V_{CO2}, respiratory exchange ratio (RER) and energy expenditure (EE) while mice completed 2 days of the Free task followed by 2 days of the FR1 task for the 20mg grain pellets (Fig 3A). As in our prior experiments, mice took ~30% fewer pellets in the FR1 vs.

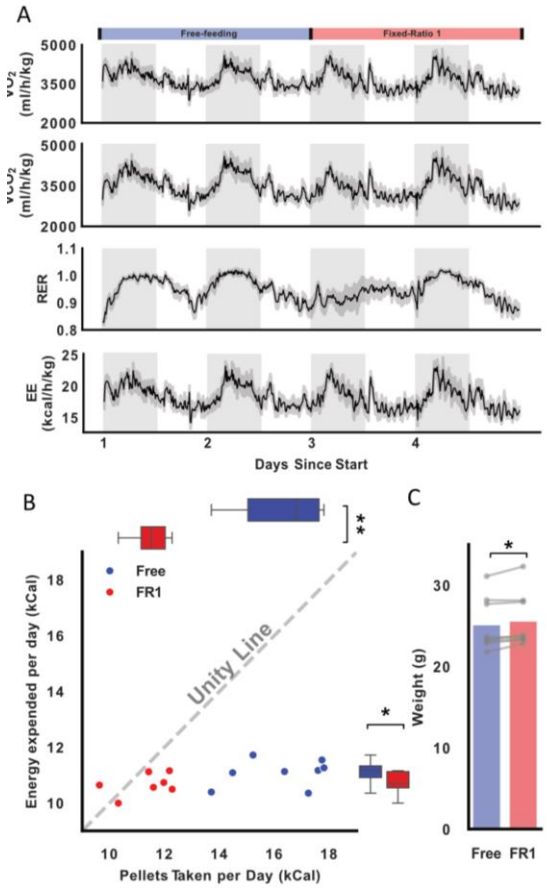

Figure 3: Mice expend ~4% more energy on Free vs. FR1 tasks. A. VO2, VCO2, RER and energy expenditure (EE), from mice (n=8) completing two days each of Free and FR1 tasks. **B**. Quantification of total energy expended vs. pellets taken in Free vs. FR1 tasks. **C**. Body weight of mice at completion of Free vs. FR1 tasks. ** denotes p<0.001 * denotes p<0.05.

Free phases during the indirect calorimetry experiments (average Kcal taken: 16.3 in Free vs 11.4 in FR1, paired t-test p<0.001, Fig 3B). In the FR1 phase, mice expended roughly the same number of calories as they took (Kcal taken: 11.4, expended: 10.7, p = 0.08, Fig 3B) while taking far more than they expended in the Free phase (Kcal taken: 16.3, expended: 11.1Kcal, p<0.001). Mice also experienced a slight weight gain on the FR1 phase relative to the Free phase in this experiment (average weight after Free: 25.2g, after FR1: 25.7g, p<0.05, Fig 3C), which does not explain the large number of excess pellets taken in the Free phase. Overall, mice expended ~4% more calories in the Free phase, suggesting they may have consumed slightly more calories in the Free phase relative to the FR1 phase. However, as this does not explain the majority of excess calories taken, we assume that mice discard many of the excess pellets they take in the Free task.

To directly measure if mice discarded more food in the Free vs. FR1 tasks, a new group of 11 mice (8M/3F) completed the Free and FR1 tasks with a white “iso-pad” paper bedding that allowed us to count pellets left over each day (Fig S3A-B). Again, mice took more pellets in Free vs. FR1 (paired t-test, p<0.001). An average of 13 pellets (~7% of daily pellets taken) were left over in each cage after the Free task, while no extra pellets were observed after the FR1 task (paired t-test, p = 0.029). While this still did not fully account for the difference in pellets taken in the Free vs. FR1 task, it demonstrates that mice remove more pellets without eating them in the Free, but not the FR1 task. Even with our efforts to measure left-over food, it is likely that mice grind up and spill food in ways that were difficult for us to quantify. From this data we conclude that mice are likely less efficient eaters in the Free task vs. the FR1 task, resulting in more spilling and grinding of food (24).

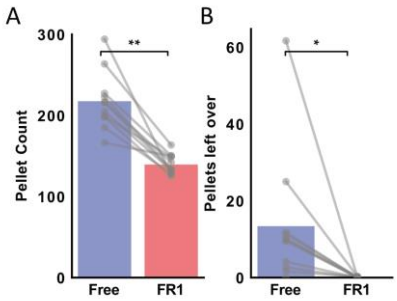

Figure S3: Mice hoard ~5% more pellets on Free vs. FR1 task. A. Average daily pellets taken on Free vs. FR1 tasks (n=11 mice, 5 days each). **B**. Average pellets left over (ie: hoarded) each day. ** denotes p<0.001 * denotes p<0.05.

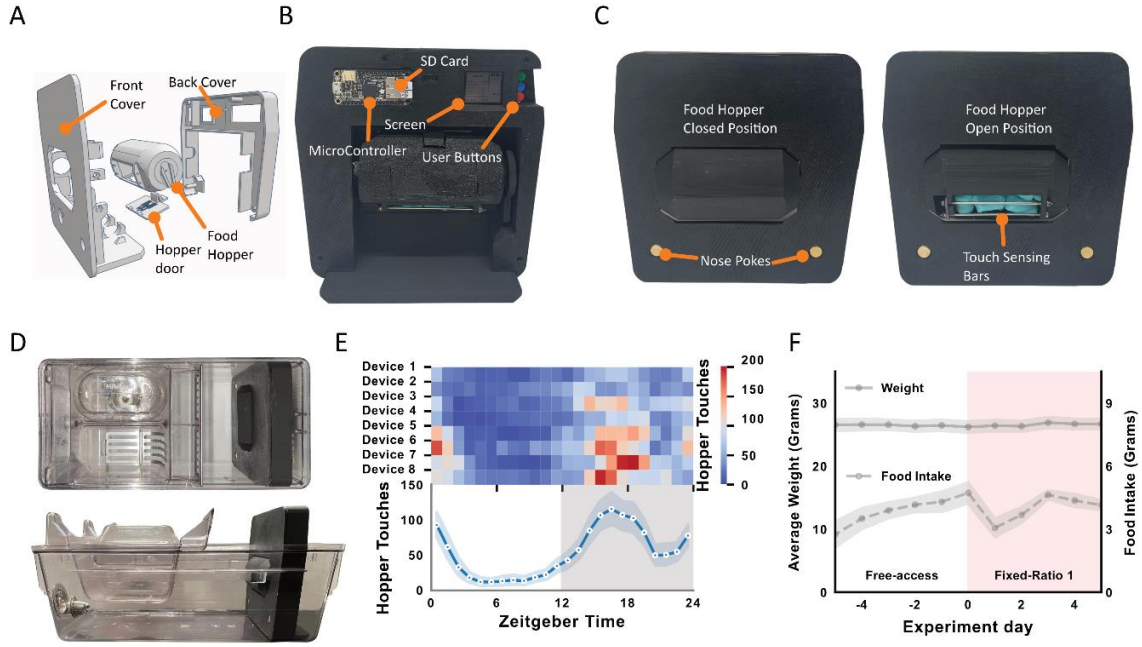

Figure 4: Design of the Tumble Feeder, a new device to control access to HFD on Free and FR1 tasks. A. 3D design of the Tumble Feeder. **B**. Back view of the assembled Tumble Feeder with relevant parts labeled. **C.** Front view of the Tumble Feeder in both open-and closed positions. **D**. Photographs of Tumble Feeder in a mouse home cage. **E**. Average daily circadian interactions with the Tumble Feeder hopper (n=8 mice over the eight days, interacting for laboratory chow). **F**. Average weight and food intake of mice completing Free vs. FR1 tasks for laboratory chow (n=8).

An operant device for controlling access to high-fat diet

High-fat diet is commonly used to model obesity in rodents, as it induces them to overeat and gain weight (14,15). Based on our findings with low-fat grain-based food pellets, we hypothesized that requiring a single nose-poke may also attenuate intake of high-fat diet and prevent high-fat diet induced weight gain. However, due to the soft composition of high-fat diet, we could not use FED3 to dispense it. To overcome this limitation, we developed a novel open-source device to control access to high-fat diet in the home-cage (termed: TumbleFeeder, design files available at https://github.com/KravitzLabDevices/CastleFeeder/, Fig 4A-D). The Tumble Feeder has two touch-sensitive nose-poke triggers for the mouse to interact with, touch sensitive bars to detect food hopper interactions, and a microcontroller, screen, real-time clock (RTC), and micro secure-digital (microSD) card slot for controlling tasks and displaying and

logging data (Fig 4B, C). We programmed the Tumble Feeder so the hopper could be left open continuously (ie: Free) or open for 60s every time the mouse touched a nose-poke (ie: FR1). In both modes, mice were allowed to access the hopper an unlimited number of times per day, with no timeout between nose-pokes in the FR1 mode. To test the performance of the Tumble Feeder with normal chow, eight mice were given access to the Free task for 5 days, resulting in the expected circadian rhythm of food hopper interactions (Fig 4E). The Tumble Feeders were then switched to FR1 mode for an additional 5 days, during which mice had to touch a nose-poke to open the food hopper to access chow for 60s. Mice rapidly learned to operate the Tumble Feeder on FR1, as evidenced by an increasing number of pokes per day (Fig S4A). Mice also maintained their body weights during both tasks, again confirming that mice could operate the Tumble Feeder to obtain their necessary daily caloric requirements in both Free and FR1 modes (Fig 4F).

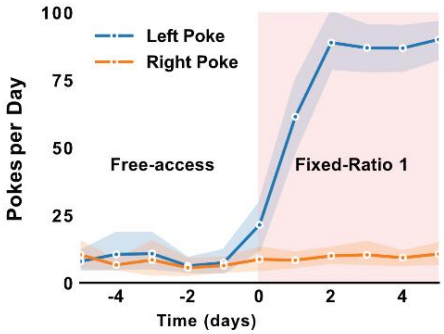

Figure S4: Mice learn to poke on the correct side to obtain access to chow. Number of pokes made per day in the chow phase between free feeding & FR1 (n=8 mice, 5 days in each phase).

Does a single nose-poke reduce high-fat diet intake and weight gain?

To test if requiring mice to nose-poke to obtain access to high-fat diet would reduce high-fat diet intake and weight gain, 12 male mice were first given access to the Tumble Feeder for 5 days with laboratory chow in the hopper. This allowed the mice to acclimate to eating from the Tumble Feeder and allowed us to quantify daily intake of Chow before starting the high-fat diet experiment. Next, the Tumble Feeder was programmed to deliver alternating 5-day phases of Free and FR1 (Fig 5A, B) for high-fat diet (60% of calories from fat, Research Diets #D12492, Movie S1), with each nose-poke providing 60s of access to high-fat diet in the FR1 phases.

Surprisingly, mice only gained weight in the Free phases, and lost a small amount of weight in the FR1 phase (Fig 5A, B). The average weight gain during the Free phases was 3.09 grams, and the average loss during the FR1 phase was 1.09 grams (Significant effect of Task $F(2, 22) = 85.9$, $p < 0.001$, post-hoc paired t-test between Free and FR1, $p < 0.001$). The Tumble Feeders were also weighed daily throughout the experiment to quantify how much high-fat diet was removed from the device each day. Consistent with the difference in weight gain, mice removed significantly more food in the Free phase (Free: 19kcal/day vs. FR1: 11kcal/day, paired t-test $p<0.001$, Fig S5C). We repeated this experiment with 8 female mice, finding similar effects on body weight and food intake (Figure S5A, B)

We next tested if requiring a nose-poke to obtain high-fat diet would prevent the development of obesity over several weeks of high-fat diet exposure. Male mice were used in this experiment as female mice are more often resistant to high-fat diet induced weight gain (25). A group of 7 male C57Bl6 mice were tested on the FR1 task to obtain high-fat diet for 6 weeks. Despite eating all daily calories from high-fat diet, these mice exhibited minimal weight gain over the 6 weeks, which did not differ significantly from the expected growth curve of age-matched male C57Bl6 mice (data from Jax, dashed line on Fig 5C, one-sample t-test $p = 0.138$). Importantly, there was no restriction on how much high-fat diet mice ate, provided they nose-poked once to open the hopper for 60s each time they wanted access. The average number of nose-pokes per day in the FR1 task was ~50, meaning the hopper was open for an average of ~50 minutes per day. As such, there was ample time (~23 hours/day) for the mice to nose-poke more to obtain more high-fat diet. At the conclusion of this experiment, the Tumble Feeders were switched to the Free mode for 6 additional weeks, which resulted in a rapid increase in body weight (Fig 5C, D), confirming that the FR1 task was suppressing high-fat diet-induced weight gain (weight gain in FR1: 7.9 grams, in Free: 19.4 grams, paired t-test $p<0.001$). This supports our hypothesis that a necessary component of the high-fat diet-induced obesity

paradigm is unrestricted access to high-fat diet, and requiring mice to perform a single action is sufficient to prevent weight gain.

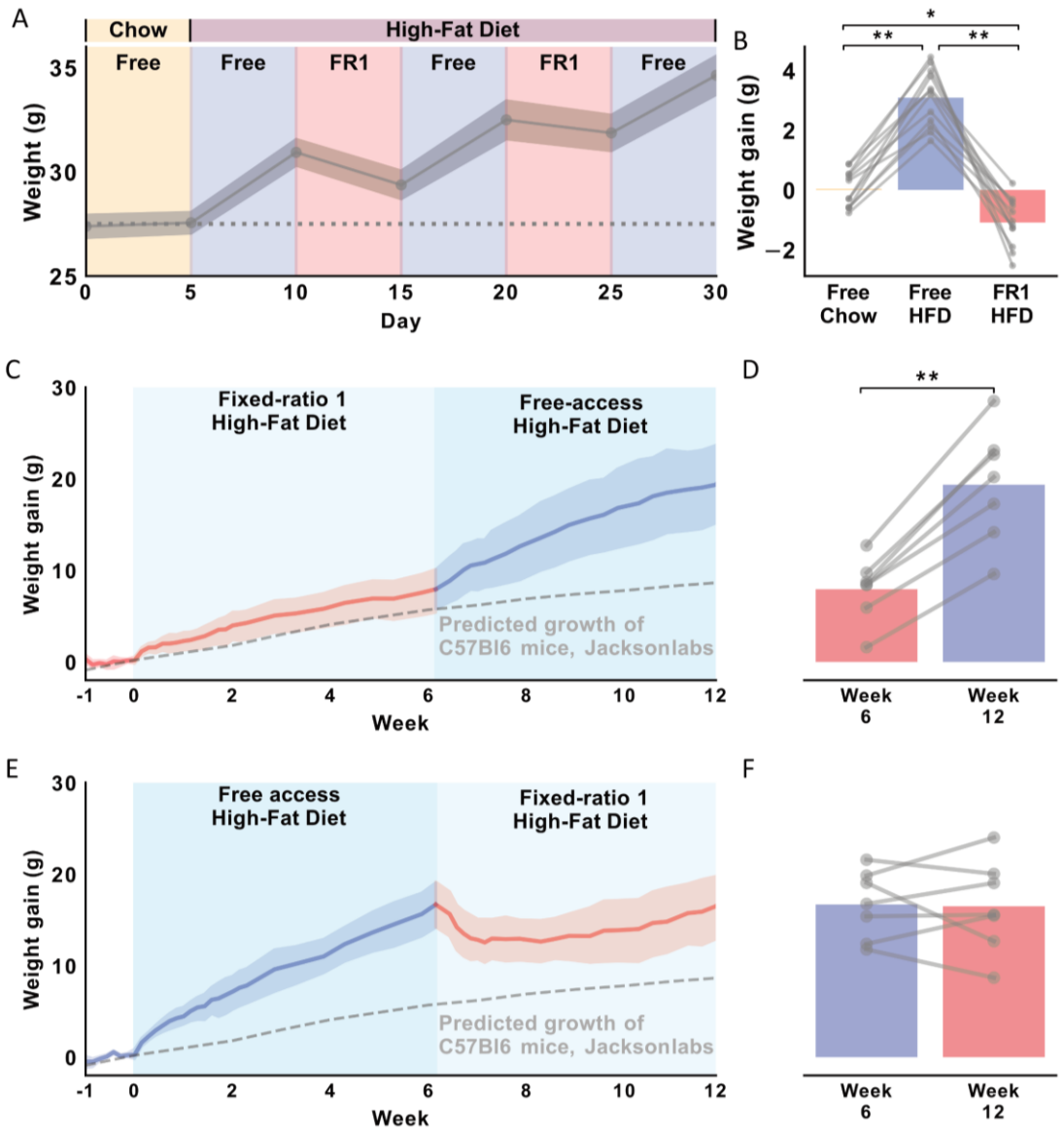

Figure 5: Mice take less high-fat diet (HFD), and gain less weight, when it is delivered on FR1 vs. Free access. **A**. Average weight of mice over a 30-day experiment where the task requirement and outcome changed every 5 days (n=12 male mice). **B**. Average weight gain of males during each phase of the experiment. **C**. Average weight of mice that were maintained on FR1 with high fat diet for 6 weeks, and then switched to Free access for six weeks (n=7 male mice). Dashed line is predicted growth curve of age-matched C57Bl6 mice from Jackson labs. **D**. Average weight at the end of the FR1 (week 6) vs. Free access period (week 12). **E**. Average weight of mice that were maintained on free acess with high fat diet for six weeks, and then switched to FR1 for six weeks (n=7 male mice). **F**. Average weight at the end of the Free (week 6) vs. FR1 access period (week 12). ** denotes p<0.001 * denotes p<0.05.

In a final experiment, we tested if requiring a nose-poke to access high-fat diet would drive weight loss in obese mice. Here, 7 new male C57Bl6 mice accessed high-fat diet from the Tumble Feeder in Free mode for 6 weeks, resulting in weight gain (Fig 5E). These mice were then switched to FR1 for 6 additional weeks. While mice lost a small amount of weight in the first week of FR1, they regained this weight over the next 6 weeks, resulting in a similar average weight at the start and end of the FR1 phase (weight gain in Free :16.7 grams, FR1: 16.5 grams paired t-test, p = 0.90, Fig 5F). Although, they did not gain additional weight while eating high-fat diet in the FR1 phase, adding a nose-poke requirement did not drive weight loss in obese mice, highlighting how the mechanisms that control weight gain are not necessarily the same as those that can drive weight loss.

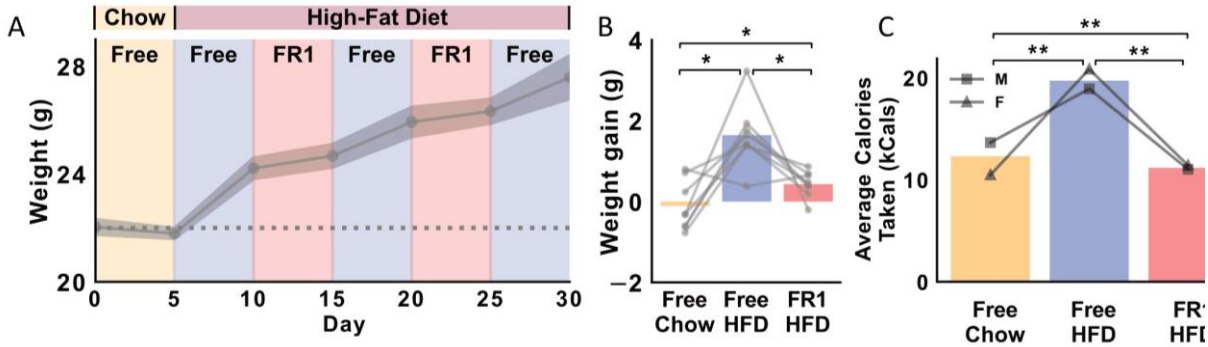

Figure S5: Mice take less high-fat diet (HFD), and gain less weight, when it is delivered on FR1 vs. Free access. A. Average weight of mice over a 30-day experiment where the task requirement and outcome changed every 5 days (n=9 female mice). **B**. Average weight gain of mice during each phase of the experiment. **C**. Average calories taken in the free chow, free HFD and FR1 HFD tasks between male and female mice. ** denotes p<0.001 * denotes p<0.05.

Discussion

Here, we found that introducing a small requirement – one nose-poke – reduced how much mice interacted with and consumed both grain-based pellets and high-fat diet, and importantly attenuated high-fat diet induced weight gain. Our results may explain how increased access to unhealthy foods have contributed to the obesity epidemic over recent decades (5,7,13), and may inform new solutions for reducing obesity rates by making such foods harder to access (26,27). However, we also found that requiring obese mice to nose-poke for access to high-fat diet did not lead to weight loss, highlighting how distinct strategies are needed for preventing and reversing obesity.

Nudging to combat obesity

Strategies that reduce overall food intake are important for lowering obesity rates (2–4). One idea for how to reduce food intake builds on the “nudge” theory of behavior (28), which suggests that small changes in the environment that make foods less convenient to obtain can reduce consumption (29). In 1968, Nisbett demonstrated this effect by offering sandwiches to people of normal weight or people with obesity. The sandwiches were either placed out on a table or in a refrigerator in the room (30). Despite being told they could eat as many sandwiches from the refrigerator as they liked, people with obesity ate 37% fewer sandwiches when they were placed in the refrigerator than when those same sandwiches were left out on the table. In this way, making food less convenient to obtain was effective at reducing intake in people with obesity. A recent meta-analysis determined that the position of foods impacted people’s food choices in 16 out of 18 studies analyzed, “nudging” participants towards healthier options by changing their location or proximity to the participant (31). While it is clear that “nudging” can alter food choices and consumption over short durations in laboratory tests, it is less clear if this approach can be used over longer durations to alter body weight (29).

To address this challenge we turned to mice, as we can sustain changes in their environment over multiple weeks. We used both an existing pellet-dispensing device (FED3) to dispense grain-based pellets and designed a novel device (the Tumble Feeder) to control access to high-fat diet, to test whether requiring mice to perform an action (one nose-poke) would reduce food intake and weight gain. Surprisingly, requiring mice to perform a single nose-poke greatly attenuated intake of both grain-based pellets and high-fat diets, and reduced high-fat diet induced weight gain, relative to when those same diets were freely available. In this way, our results demonstrate how a small change in the environment can reduce food intake and slow the development of obesity in mice, provided the change is sustained over multiple weeks.

Why did mice take less food when they had to nose-poke to get it?

We considered why mice took less food when they had to perform one nose-poke, vs. when the same food was provided freely. We first considered if the timing of food intake might explain this, as mice took more time between each pellet when they had to perform a nose-poke, which might allow more time for satiety signals to reach the brain (23). However, artificially slowing down eating rates in the free access condition did not reduce intake, suggesting that this was likely not the explanation. We also considered that mice might not understand the nose-poking task well enough to efficiently food. However, this also did not appear to be a sufficient explanation, as the mice achieved 80% accuracy on the FED3 nose-poking task within 4 hours, and averaged close to 200 pellets per day on this task. Moreover, mice exhibited this pattern even with multiple cycles of FR1 and free feeding, and when the FR1 task was made even easier by delivering food access in response to either of the two pokes. Finally, we considered whether the physical effort of nose-poking was causing mice to avoid taking excess food. Again, this does not seem like a plausible explanation, as we only required them to poke one time to receive food, and mice can obtain enough pellets to maintain their body weight even when they have to poke 50 times to receive each food pellet (32).

Out of sight, out of mind: the impact of cue-induced feeding on food intake

A clue to the reduction in intake in our nose-poking task may be gleaned from the temporal structure of their pellet taking. When we broke their daily feeding into “meals”, we found that mice initiated 39% fewer meals when we required them to nose-poke, compared to when the food pellets were freely available. This was the largest change in their feeding patterns, and suggests that the direct access to the sight or smell of the food, induced mice to initiate more bouts of feeding. This may give insight into the neural mechanisms that underlie why requiring a nose-poke reduced food intake over freely available food.

For example, several researchers have noted that cues that predict food are sufficient to make animals seek food, even when sated, a phenomenon known as “cue induced feeding” (33,34). Reppucci et al. trained rats to associate a tone with delivery of a food pellet, with control animals receiving the same tones unpaired from food pellet delivery. They then played those tones to the rats in their home-cages for 5 minutes, before giving them the option to eat food pellets or regular laboratory chow for 4 hours. The rats that had learned to associate the tone with the food pellets ate ~63% more pellets in the consumption test than the control animals, demonstrating that food-paired cues were sufficient to induce over-eating, at least on the time scales recorded in this study. As with auditory cues, the sight or smell of food may serve as additional sensory cues that induce animals to seek and take food. In this way, keeping food sequestered inside of the FED3 or Tumble Feeder devices until the mouse performed a nose-poke may have reduced the salience of the sensory cues from the food and thereby reduced food seeking and taking.

Through lesion studies, cue-induced feeding has been shown to rely on the amygdala, hippocampus, prefrontal cortex, and lateral hypothalamus (35). Hypothalamic arcuate nucleus agouti-related peptide (AGRP) and pro-opiomelanocortin (POMC) neurons are also rapidly modulated by the sensory detection of food, which may contribute to subsequent consumption

(36). In the free-feeding condition, animals likely investigate the food even when not hungry, simply because they have unrestricted access to it and little else to do in their cages. This may have impacted the activity of AGRP and POMC neurons to drive further feeding (37,38). Follow up studies could test the involvement of specific brain circuits or cell types on the behavioral effects we observed. For instance, a brain manipulation that normalized the difference in pellets taken in the Free vs FR1 tasks could inform future brain-based approaches to reduce food intake in food-rich environments.

Does making high-fat diet harder to get reverse obesity?

In our final experiment we tested whether requiring a nose-poke to obtain high-fat diet would induce weight loss in obese mice. The obese mice continued to obtain all their daily calories from high-fat diet, but they had to nose-poke once to obtain access to the high-fat diet for one minute each time they wanted to eat. While obese mice did not gain additional weight once the FR1 requirement was introduced, they remained at their elevated body weight and did not lose weight. This highlights the challenges of weight loss, and how obese animals and people will defend their elevated body weights through multiple mechanisms (39,40). Overall, our results suggest that making foods harder to obtain may help prevent, but not reverse, obesity in modern food environments.

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Methods

Mice and husbandry. C57BL6 mice were individually housed for each experiment on a 12h light/dark cycle. Food was provided as specified in the results and included 20mg grain-based food pellets (Bio-Serv F0163), 20mg sucrose pellets (Bio-Serv F07595), laboratory chow, and 60% high-fat diet (Research Diets #D12492). Water was available *ad libitum* throughout the experiments. All procedures were approved by the Animal Care and Use Committees at Washington University in St Louis, The Artic University of Norway, Monash University, Rutgers University, Temple University, the National Institute of Health, Williams College, Washington State University and Swarthmore College.

Behavioral testing: FED3

The FED3 electronics and hardware have previously been described (Matikainen-Ankney 2021). FED3 is a small battery-powered pellet dispensing device, which was placed in the home-cage so mice were able to interact with it around-the-clock for multiple days. FED3 saves all data on a microSD card for later analysis. There was no limit to the number of pellets the mouse could earn from FED3, which provided 20mg food pellets to mice based on tasks described below. In all tasks, we defined meals as pellets eaten within 2 min of each other, based on inter-pellet interval histograms. In addition, we defined a minimum size of 0.06 g (three pellets) to be counted as a meal.

Free-feeding (Free): In the Free task, FED3 started up with a pellet in its pellet receptacle, and it replaced this with a new pellet 5s after the mouse removed it. FED3 logged the times of all pellet retrieval events for later analysis.

Free-feeding with time-out: In a variant of the Free task, FED3 started up with a pellet in its pellet receptacle, and waited 35 seconds before replacing it with a new pellet when the mouse removed it. FED3 logged the times of all pellet retrieval events for later analysis.

Fixed-ratio 1 (FR1): In FR1 mode, the mouse had to break the left beam-break trigger with its nose (ie: a nose-poke) to cause FED3 to dispense a pellet. After the mouse removed the pellet, it had to nose-poke again to dispense another pellet. There was no time-out period following each pellet delivery, and mice could poke and earn an unlimited number of pellets. However, the nose poke became inactive whenever there was a pellet in the feeding well to prevent buildup of pellets. FED3 logged the times of all nose-poke and pellet retrieval events for later analysis.

Fixed-ratio 1 (dual-FR1): In dual-FR1 mode, the mouse had to break either the left or right a beam-break trigger with its nose to cause FED3 to dispense a pellet.

Fixed-ratio 3 (FR3): In FR3 mode, the mouse had to break the left beam-break trigger three times with its nose to cause FED3 to dispense a pellet.

Behavioral testing: Tumble Feeder

The Tumble feeder is an open-source hopper-based device that can be used in the home-cage with mice to control their access to food over multiple weeks.

3D design. The 3D parts for FED3 were designed with TinkerCAD (Autodesk). Tumble Feeders were printed in house with an FDM printer in PLA (Bambu X1 Carbon). We provide editable design files here: https://www.tinkercad.com/things/1IhfIT78Xpz-tumble-feeder-sept2024.

Electronics. The Tumble Feeder has two capacitive touch-sensitive nose-poke triggers for the mouse to interact with, a servo-controlled hopper (Analog Feedback Micro Servo with metal

gear, Adafruit #1450), touch sensitive bars (UXCELL M2x65mm Stainless Steel Pushrod Connectors) to detect food hopper interactions via capacitive touch sensing, and uses a microcontroller with micro secure-digital (microSD) card slot for controlling tasks, and a Sharp Memory Display (Adafruit #3502), and a DS3231 Precision real-time clock (RTC, Adafruit #3028) for displaying and logging data. The Adalogger M0 contains an ATSAMD21G18 ARM M0 processor that runs at 48 MHz, 256 kB of FLASH memory, 32 kB of RAM memory and up to 20 digital inputs/output pins for controlling other hardware. The microcontroller also contains a battery charging circuit for charging the internal 2200mAhr LiPo battery in FED3, which provides ~1 month of run-time between charges. The exact battery life depends on the behavioral program and how often the mouse triggers the Tumble Feeder to open as the Servo motor is the main consumer of power. Electronics were programmed in the Arduino IDE and the code is available at https://github.com/KravitzLabDevices/CastleFeeder/.

Behavioral tasks. The Tumble Feeder is battery-powered and was placed in the home-cage so mice were able to interact with it around-the-clock for multiple days. The Tumble Feeder was filled with either laboratory chow or high-fat diet, as described in the results, and saved touch times and hopper interaction times on a microSD card for later analysis. The Tumble Feeder was programmed to deliver food based on the task requirements described below:

Free-feeding (Free): In Free-feeding mode in the tumblers were open to allow ad libitum access to food. The touch-sensitive nose-poke triggers had no programmed consequences, and time stamps of touching the metal bars on the hopper were logged to the internal microSD card for later analysis. Every fifteen minutes (96 times per day), the hopper would close and re-open, to control for the noise and movements that occurred in the FR1 session.

Fixed-ratio 1 (FR1): In FR1 mode, the hopper was normally closed and required the mouse to contact the left touch sensor to open it. If the mouse contacted the Left touch sensor the hopper would open for 60s. There were no limits to the number of times the mouse could open the hopper each day, and there were no time-out periods following each time it closed. Time stamps of nose-poke contacts and touching the metal bars on the hopper were logged to the internal microSD card for later analysis.

Indirect calorimetry

A PhenoMaster metabolic cage system (TSE Systems) was used to measure oxygen consumption (V_{O2}), carbon dioxide production (V_{CO2}), respiratory exchange ratio (RER), and Energy Expenditure (EE) in animals completing Free-feeding and FR1 with FED3. The FED3 devices were battery powered and placed entirely within the PhenoMaster caging with no wiring that might interfere with air handling. Mice were previously acclimated to FED3 and were placed in the PhenoMaster for 2 days with FED3 in Free mode, after which FED3 was switched to FR1 mode for an additional 2 days of monitoring.

Data analysis and statistics

CSV files generated by FED3 and Tumble Feeder were processed with custom python scripts (Python, version 3.6.7, Python Software Foundation, Wilmington, DE). Data wrangling and plotting used Pandas (41), Matplotlib (42), Pingouin (43), and Seaborn (44) packages. One- or two-way ANOVAs with post-hoc t-tests or paired or unpaired t-tests were used to compare groups as appropriate. p-values < 0.05 were considered significant. Data sets are presented as mean ± SEM. Numbers of animals per experiment is listed as n=number of animals. ChatGPT 4o was used for assistance with coding, but the Authors take full responsibility for all analysis code. All data and analysis and visualization code are available on Github (https://github.com/KravitzLab/Barrett2024).

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