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Citric acid water as an alternative to food restriction to motivate task performance in mice during touchscreen testing

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Rodent behavioral testing paradigms in touchscreen operant chambers have successfully provided insight into the neural mechanisms underlying various cognitive domains in healthy and disease models. Touchscreen testing has previously required food restriction to sufficiently motivate rodents to complete behavioral tests, limiting the use of interventions, for example, diet-based interventions, that alter animals' motivation for food in experimental design. Here we explored the safety and efficacy of water manipulation via the addition of citric acid in motivating behavioral performance in touchscreen operant chambers (1) in comparison with food restriction and (2) when mice are fed an obesogenic high-fat, high-sugar (HFHS) diet. Water manipulation and food restriction produced similar performance on the progressive ratio task in nonobesogenic, standard-fed mice. However, when water-manipulated mice were fed an HFHS diet, they showed deficits in this motivation-sensitive task compared with standard chow-fed mice. Critically, all groups, regardless of restriction type or diet, showed similar learning curves during a pairwise visual discrimination task. Together, these findings demonstrate that water manipulation can safely and effectively motivate mice to perform touchscreen tasks for reward, even when fed a highly satiating HFHS diet, which opens the possibility of using interventions, especially diet-based interventions, in conjunction with touchscreen cognitive testing batteries.

Advancements in the understanding and treatment of human diseases are largely due to the use of preclinical experimental models. Preclinical models permit direct manipulation and observation of factors underlying putative disease states[1,2]. In neuroscience research, studies using rodent models have provided substantial insight into the mechanisms underlying many neurological conditions[2]; however, many of these study methods involve tests of cognition that are not directly translatable to human populations[3–6]. Automated touchscreen operant chambers offer several advantages to traditional hand-testing tasks by minimizing experimenter interference, using standardized operating procedures and enabling cross-species investigations through a visual-based modality. Tasks specifically developed for touchscreens permit a greater level of control and translatability by administering virtually identical and visual-based paradigms to both rodent and human subjects[4–7]. Studies with human participants increasingly use computerized test batteries, including CogState, Mindstreams and the Cambridge Neuropsychological Test Automated Battery (CANTAB), among others[8]. Rodent touchscreen operant chambers use similar principles, bridging the gap between rodent research and behavioral assessments in humans[9]. The rate of uptake and publication of experiments using the rodent touchscreen has increased dramatically (currently over 800 publications).

Rodent touchscreen paradigms, similar to many other behavioral paradigms, require the use of an appetitive reinforcer (reward), such as a strawberry milkshake[7,9,10]. Food or liquid rewards avoid the use of aversive stimuli, removing stress or pain-induced conditioned responding in mice[11]. This approach motivates spontaneous animal behavior and supports animal welfare[11]. Motivation to complete tasks with caloric reinforcers (such as the strawberry milkshake that is the most common

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Table 1 | Health scoring rubric used to assess activity levels and hydration of mice on CA water

Activity	Score
Moves around the cage	0
Moves slowly around the cage	1
Moves only when touched	2
Does not move	3
Posture and grooming	
Normal posture and smooth fur	0
Hunched posture or ruffled fur	1
Hunched posture and slightly ruffled fur	2
Hunched posture and all fur ruffled	3
Signs of eating and drinking	
Feces and urine observed	0
Minimal feces and/or urine	1
No signs of feces and/or urine	2
Signs of dehydration	
Skin does not tent when scuffed	0
Skin tents briefly but returns to normal	1
Skin tents and takes more than 2 s	2
Skin tents and stays tented	3
Total scores Any animal that has a score of: • ≤4 cumulatively or ≤1 in any one category should be monitored but no action required • ≥2 in any one category or cumulatively ≥5 required veterinary support to monitor the animal	

Mice undergoing water manipulation treatment were monitored using this table, six times per week.

reinforcer in touchscreen operant experiments) is usually enhanced through food restriction, which consists of limiting food access by duration or quantity[12–14]. The need for food restriction is a substantial limitation in studies that may alter an animal's motivation for food or liquid rewards as part of a dietary experimental manipulation, such as the cuprizone diet in studies of demyelination or the high-fat, high-sugar (HFHS) diet in studies of diet-induced obesity[15,16]. Specialized diets often require ad libitum availability to produce the effects of the diet in a way that are translatable to humans[17,18]. For example, diet-induced obesity models require ad libitum access to HFHS or other obesogenic diets to model the ease of access and the convenience of westernized diets. However, diet-induced obesity is highly demotivating in tasks that incorporate an appetitive reinforcer[16,19,20] and ad libitum access to even standard laboratory chow is sufficient to demotivate mice and reduce responding in touchscreen testing[21]. An alternative protocol is thus required to maintain the desirability of an appetitive reinforcer so that rodents on diet manipulations can be motivated to perform touchscreen tests of cognition.

Water restriction is one such alternative to food restriction. Water restriction has been applied in two different ways: by limiting the duration of access to water[22] or by limiting the amount of water that is accessible to the rodent[23]. Previous studies have demonstrated that water restriction protocols in animals not undergoing food restriction effectively increase motivation in touchscreen and operant paradigms that use liquid reinforcers[12,19]. Despite its efficacy, water restriction presents greater health risks than food restriction and requires rigorous monitoring to ensure the health of the rodents[12] (Table 1).

Water manipulation differs from water restriction in that it does not limit the availability or quantity of water[24]. Water manipulation occurs through the addition of a small amount of citric acid (CA) to ad libitum drinking water, which creates a solution with a mild sour taste[25] and in turn reduces the quantity of water that mice consume[24,25]. This reduction in water consumption is sufficient to motivate rodents to successfully complete behavioral conditioning paradigms when presented with water rewards, with a reduced risk of dehydration in comparison with other water-restriction protocols[24,25].

Water manipulation using CA has previously demonstrated efficacy in motivating rodents to complete a test of learning in standard operant chambers where water was used as the appetitive reinforcer. However, these studies did not include an explicit test of motivation nor did they use a caloric reinforcer. This study aimed to investigate (1) whether mice undergoing water manipulation perform similarly to food-restricted mice on touchscreen tasks of motivation and discrimination learning in which strawberry milkshake is the appetitive reinforcer and (2) whether mice consuming an ad libitum HFHS diet are as motivated as mice consuming standard chow to complete touchscreen tasks (in which strawberry milkshake is the appetitive reinforcer) while undergoing the water-manipulation protocol. Mice were evaluated in terms of motivation (using touchscreen fixed ratio (FR) and progressive ratio (PR) tasks), learning (using a touchscreen pairwise visual discrimination (PVD) task) and health outcomes. In addition, sex was considered in this study, as male and female mice respond differently to changes in diet, including body weight and volume of diet consumption[26].

Results

Experimental design

Three experimental conditions were used (Fig. 1). All three conditions included male and female C57BL/6J mice. The mice in condition A were food restricted on standard chow to 85–90% of their free-feeding body weight with no water manipulation (that is, unrestricted access to tap water). Mice in condition B were fed an ad libitum standard chow diet while on the water-manipulation protocol (consuming CA water). Finally, mice in condition C were fed an ad libitum HFHS diet while on the water-manipulation protocol (consuming CA water). Including a group with food restriction and an ad libitum HFHS diet was of course not possible. Previously, Yang et al.[21] established the importance of food restriction in motivating mice to complete touchscreen tasks by demonstrating decreased response rates and interactions with touchscreens when mice were returned to ad libitum feeding following testing while on food restriction. Decades of operant testing have used food restriction as a motivator, as free-fed animals fail to advance through pretraining, let alone training and testing. As such, free-feeding mice that were not undergoing water manipulation were not used in the current study, as they would be unable to produce meaningful data and would result in using animals for little benefit (in contravention of the 3Rs (replacement, reduction and refinement) of animal research)[27]. Thus, our analyses are based on two different comparisons of these three conditions: (1) the standard-diet comparison, comparing conditions A and B; and (2) the water-manipulation comparison, which compares conditions B and C. The aim of comparison 1 is to establish whether water manipulation motivates mice as well as food restriction, whereas the aim of comparison 2 is to establish whether water manipulation is sufficient to motivate touchscreen task performance in mice fed ad libitum a highly appetitive HFHS diet known to decrease responding in appetitive operant tasks[28]. In consideration of the 3Rs, mice in condition B were the same in both comparisons.

Mice subjected to water manipulation complete the FR and PR task with similar levels of motivation as food-restricted mice

Mice were habituated to the touchscreen chambers and strawberry milkshake reward over 3 days before undergoing a series of pretraining stages to interact with the touchscreens. To train and progress to PR, mice moved through FR reward schedules, each requiring a higher number of responses to earn rewards (Methods; full standard operating protocol on https://touchscreencognition.org). To address a sharp loss in weight in water-manipulated groups, before touchscreen testing, the CA concentration was reduced from 2% to 1.5% (Fig. 1). This change in CA

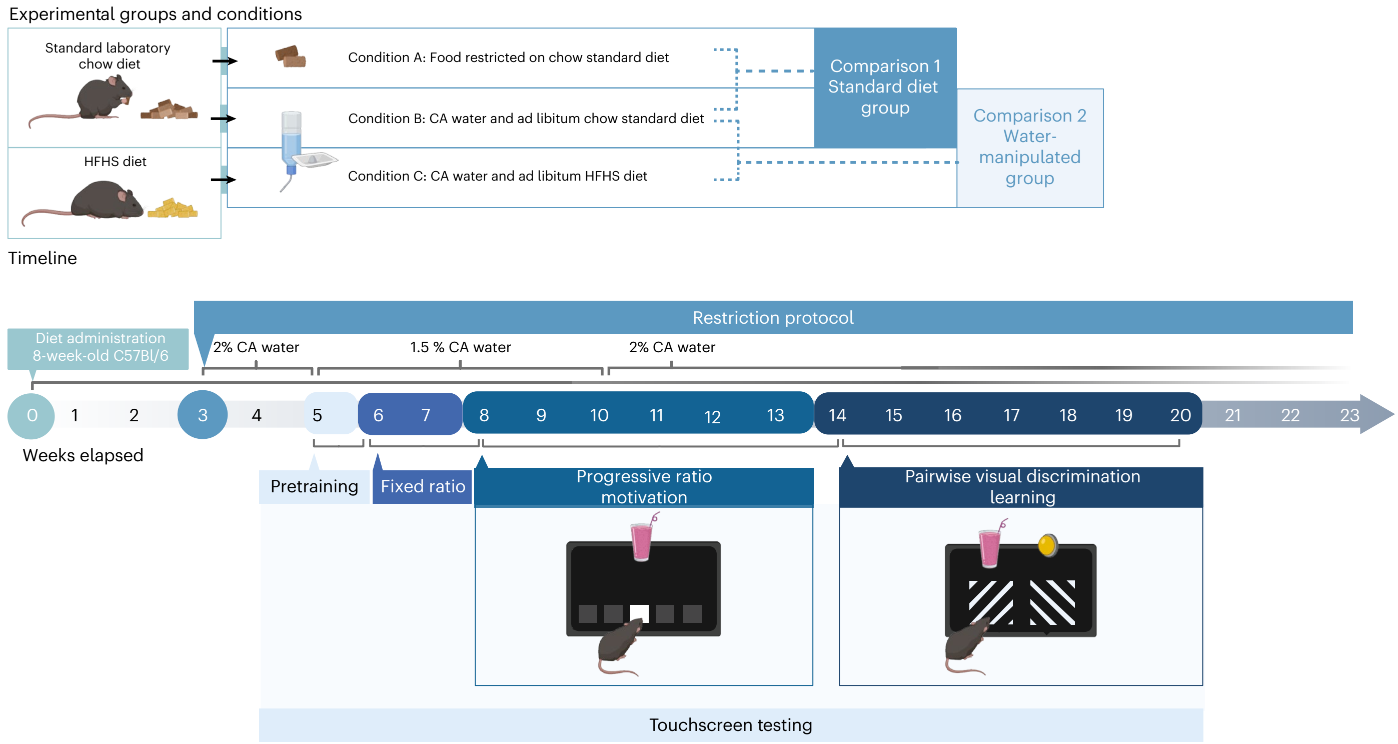

Fig. 1 | Study conditions, groups and timeline. Mice were fed either a standard laboratory chow or HFHS diet and then placed on either food restriction or water manipulation with CA water following week 3. This experimental design produced three experimental conditions. All mice were then habituated to and pretrained on touchscreen testing. Following this step, mice performed the FR and PR tasks of motivation and the PVD learning task. Weight and health were monitored throughout. Figure created in BioRender; Ghosh-swaby, O. https://biorender.com/dy50sd3 (2026).

concentration effectively stabilized body weight at 85% or greater, allowing a safe return to the 2% CA concentration without further substantial weight decline. Consequently, all mice performed FR while on 1.5% CA water only.

In the groups fed a standard diet (conditions A and B), there were no effects of restriction type or sex on sessions to criterion within the FR task (two-way analysis of variance (ANOVA), $P_s > 0.275$; Fig. 2a) for advancement to PR. Similarly, the rates of responses (that is, responses per minute) during the FR task were not affected by sex or restriction type (three-way ANOVA, $P_s > 0.275$; Fig. 2b) but increased in all groups with the number of responses required to receive reward (three-way ANOVA, main effect of reward schedule, $F_{(2.33,\ 80.383)} = 374.44$, $\eta^2 = 0.513$, $P < 0.001$; Fig. 2b). Therefore, mice in all groups progressed at a similar rate from FR to PR.

After completing the FR task, mice performed the PR test of motivation, where the number of responses required for a reward increased progressively within a reward schedule by either 4, 8 or 12 responses (that is, PR4, PR8 and PR12, respectively; Methods). To address the change in CA water concentrations from 1.5% to 2% (Fig. 1), mice performed the PR once while water-manipulated mice were on 1.5% CA water. Then, the mice performed the PR again when they returned to 2% CA water. These two runs were analyzed separately. Breakpoint, an important measure of motivation, was defined as the maximum number of responses made to receive reward. While water-manipulated mice received 1.5% CA, there were no significant differences in breakpoint between restriction types or sex (three-way ANOVA, $P_s > 0.096$; Fig. 2c, left). As expected, there was a main effect of PR reward schedule (three-way ANOVA, $F_{(1.695,\ 61.023)} = 17.630$, $\eta^2 = 0.059$, $P < 0.001$; Fig. 2c, left), where breakpoints progressively increased from PR4 to PR8 to PR12 as demands to respond were higher. To assess nonspecific or impulsive behavior, the number of blank touches (that is, responses to nontarget windows) made during image presentation were recorded. The number of blank touches made significantly decreased from PR4 to PR8 to PR12 (three-way ANOVA, $P < 0.001$; main effect of PR reward schedule, $F_{(2,\ 72)} = 8.316$, $\eta^2 = 0.036$, $P < 0.001$; Fig. 2d, left). There were no interaction effects or no main effects of sex or restriction type on the number of blank touches (three-way ANOVA, $P_s > 0.061$).

Following testing on 1.5% CA, water-manipulated mice were returned to 2% CA, and all mice were tested again on PR. There were again no effects of sex or restriction type (three-way ANOVA, $P_s > 0.051$; Fig. 2c, right) on breakpoint. Overall, mice had higher breakpoints at PR8 than PR4 and PR12, an effect driven by the males (three-way ANOVA, PR8 > PR4, $P < 0.001$; PR8 > PR12, $P = 0.004$; reward schedule-by-sex interaction, $F_{(1.374,\ 49.464)} = 4.091$, $\eta^2 = 0.011$, $P = 0.036$; main effect of PR reward schedule, $F_{(1.374,\ 49.464)} = 9.082$, $\eta^2 = 0.066$, $P = 0.002$; Fig. 2c, right). There was a main effect of PR reward schedule in the number of blank touches (three-way ANOVA, $F_{(1.713,\ 61.67)} = 9.865$, $\eta^2 = 0.066$, $P < 0.001$; Fig. 2d, right) but no effects of sex, restriction type or interaction (three-way ANOVA, $P_s > 0.117$; Fig. 2d, right).

Overall, there is no evidence that water-manipulated mice showed lower motivation to receive reward than food-restricted mice in a task highly sensitive to changes in motivation. Both restriction types produce high-yield responding during the touchscreen task, indicating the mice are motivated to seek reward regardless of restriction type.

Standard chow diet-fed mice on CA water perform similarly to food-restricted mice in a PVD task

After completing the PR task, mice performed the PVD task, in which they learned to discriminate between and respond to one of two different images presented simultaneously to receive a reward. Accuracy and the number of correction trials were recorded over 9 sessions of 30 trials each. There was a main effect of session (three-way ANOVA, $F_{(5.122,\ 184.393)} = 45.784$, $\eta^2 = 0.410$, $P < 0.001$; Fig. 3a), indicating that groups learned progressively over the nine sessions. There was also an interaction between session, sex and restriction type (three-way ANOVA,

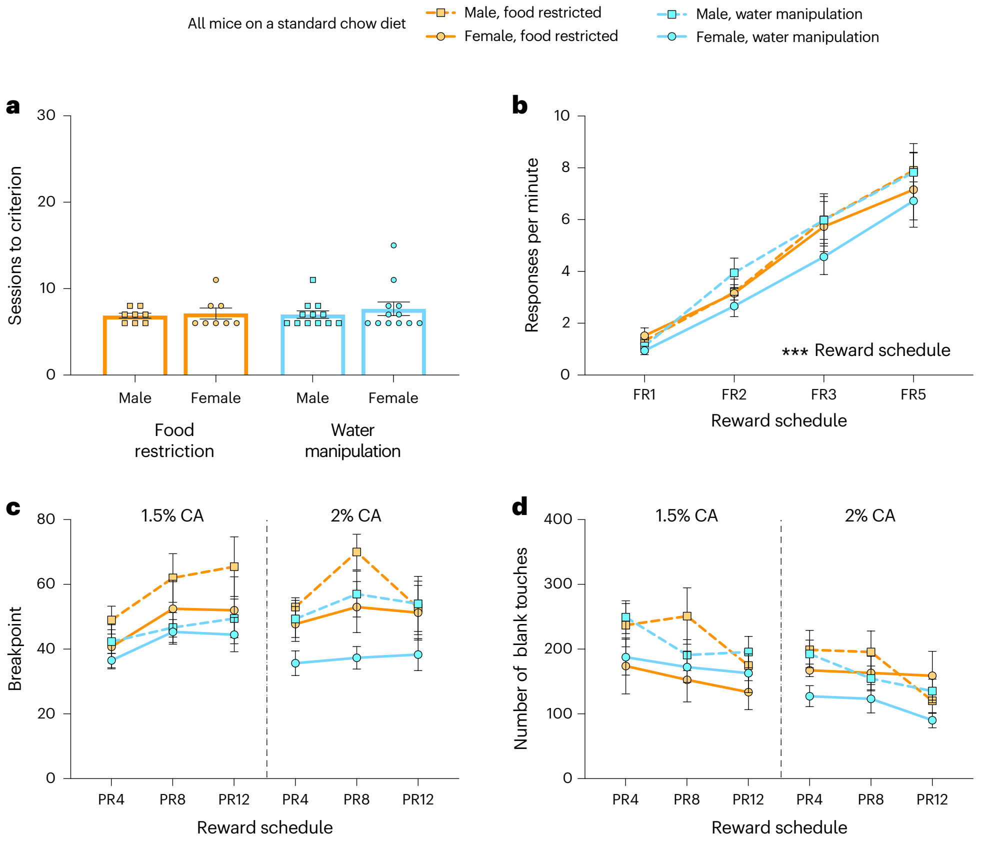

Fig. 2 | Food restriction and water manipulation produce similar levels of motivation in mice. Mice were trained on FR before advancing to PR testing. **a**, The food-restricted and water-manipulated groups did not differ significantly in the sessions required to reach criterion for advancement to PR. **b**, As expected, mice made more responses as the task demands required additional responses to earn the milkshake reward. **c**, Restriction type did not affect the breakpoint. **d**, Restriction type did not affect responses made to nonactive touchscreen windows. Sample sizes: food-restricted mice ($n = 8$ per sex); water-manipulated mice ($n = 12$ per sex). Data are presented as mean ± s.e.m., P values for main effects of three-way mixed model ANOVA; $^{***}P < 0.001$.

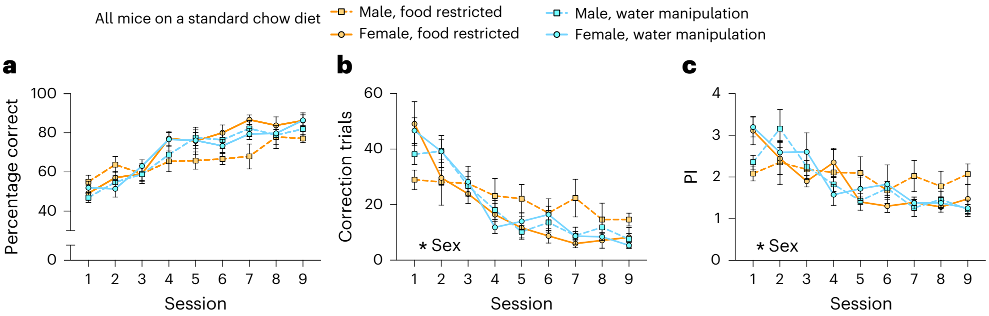

Fig. 3 | Restriction type does not affect learning in the PVD task. a–c, Restriction type did not affect the percentage of correct responding (**a**), number of correction trials (**b**) or perseverative responding (**c**) across the nine sessions of PVD, whereas there were significant main effects of sex in correction trials (**b**) and PI (**c**). Food-restricted ($n = 8$ per sex), water-manipulated ($n = 12$ per sex). Data are presented as mean ± s.e.m., P values for main effects of three-way mixed model ANOVA; $^{*}P < 0.05$.

$F_{(5.122,\,184.393)} = 2.359$, $\eta^2 = 0.021$, $P = 0.041$; no statistically significant and relevant pairwise comparisons) but no main effects of sex or restriction type (three-way ANOVA, $P_s > 0.136$). Correction trials, which occurred following an incorrect response and were repeated until the animal made a correct response but did not count toward total trial count or accuracy during the session, were also evaluated. There were main effects of session (three-way ANOVA, $F_{(4.707,\,169.439)} = 21.973$, $\eta^2 = 0.234$, $P < 0.001$; Fig. 3b) and sex (three-way ANOVA, $F_{(1,\,36)} = 6.118$, $\eta^2 = 0.043$, $P = 0.018$; males required more correction trials than females; Fig. 3b) but no main effect of restriction type (three-way ANOVA, $P = 0.932$). This result suggests that mice with CA manipulation and food-restricted mice require overall the same number of correction trials. Significant session by sex (three-way ANOVA, $F_{(4.707,\,169.439)} = 3.405$, $\eta^2 = 0.036$, $P = 0.007$) and session by restriction type (three-way ANOVA, $F_{(4.707,\,169.439)} = 2.638$, $\eta^2 = 0.028$, $P = 0.028$) interactions were observed but without relevant and statistically significant post hoc comparisons. To further contextualize these findings, perseveration indices (PIs) were calculated. The PI is a ratio of the number of correction trials to incorrect noncorrection trials, representing the number of corrections required for the mouse to complete the trial correctly. Main effects of session (three-way ANOVA, $F_{(3.946,\,142.061)} = 8.542$, $\eta^2 = 0.129$, $P < 0.001$) and sex (three-way ANOVA, $F_{(1,\,36)} = 4.800$, $\eta^2 = 0.031$, $P = 0.035$) were observed without significant effects of restriction type nor

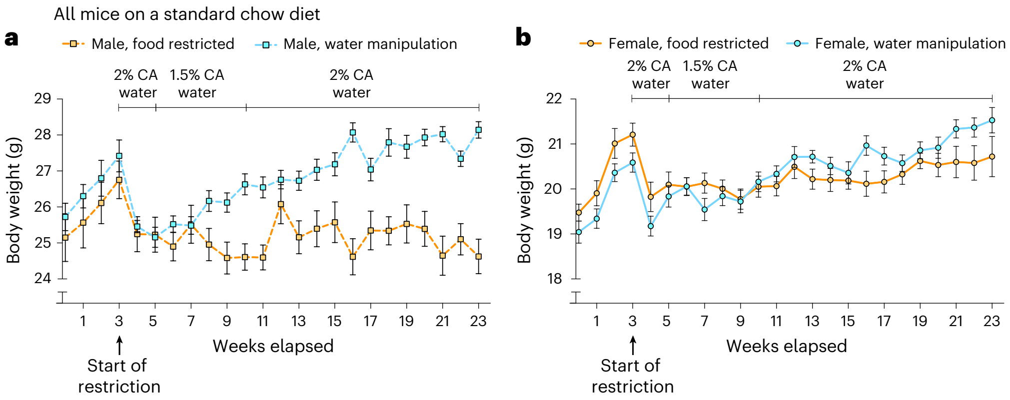

Fig. 4 | Average weekly body weight of mice fed a standard diet. a,**b**, Mice were fed standard chow ad libitum from week 0 (baseline) to week 3. Mice were weighed three times per week. Following week 3, mice started receiving either 2% CA water ($n = 12$ per sex) or began food restriction to 85–90% of their week 3 weight ($n = 8$ per sex). Mice began consuming 2% CA water on week 4 but were reduced to 1.5% CA water from week 5 to 8 to counteract body weight loss. Following weight stabilization at week 8, mice were returned to 2% CA water until the end of the study. Following week 3, mice were weighed and had their health monitored six times per week. Data are separated by sex: male data (**a**) and female data (**b**). Data are presented as mean ± s.e.m.

any interactions (three-way ANOVA, $P_s > 0.069$; Fig. 3c). Collectively, these data show that, whereas sex affected correction trial number and incorrect perseveration, restriction type did not affect PVD learning.

Mice on CA water maintain near-baseline weight and scored positively on health scores

Mice were monitored for health using a scoring rubric for categories of posture and grooming, activity levels, signs of dehydration and eating and drinking (Table 1 and Methods). Each category has a set of behaviors or conditions assigned a score from 0 (normal) to 3 (severe), with higher scores indicating signs of distress or poor health. Low scores indicated no substantial health changes within a single category (<2 out of 3) or across categories (<5 out of 11). Overall health concerns were minimal, with no mice on CA water scoring values >1 in any single health category or cumulatively. Reported scores of 1 in a single health category or cumulatively were predominantly for activity levels and signs of dehydration (that is, skin turgor test and lack of urine and feces) in both males ($n = 2$) and females ($n = 4$) during the first 4 days of CA water administration (Table 1).

Body weight was monitored throughout the experiment (Fig. 4) and analyzed following the onset of restriction. Overall, water-manipulated mice weighed significantly more than food-restricted mice (three-way ANOVA, main effect of restriction type, $F_{(1,36)} = 16.565$, $\eta^2 = 0.027$, $P < 0.001$) and male mice were heavier than female mice (three-way ANOVA, main effect of sex, $F_{(1,36)} = 516.522$, $\eta^2 = 0.827$, $P < 0.001$). There was a significant interaction between sex and restriction type (three-way ANOVA, $F_{(1,36)} = 5.236$, $\eta^2 = 0.008$, $P = 0.028$), revealing that the effect of restriction type was significant in the male mice (Holm post hoc, Cohen's $d = 1.678$, $P < 0.001$) but not in female mice (Holm post hoc, $P = 0.216$). Over the course of the experiment, mice on water manipulation gained weight following restriction whereas food-restricted mice did not (week-by-restriction type interaction: three-way ANOVA, $F_{(5.571,200.546)} = 22.889$, $\eta^2 = 0.018$, $P < 0.001$), with this being predominantly driven by the male mice (sex-by-week interaction, three-way ANOVA, $F_{(5.571,200.546)} = 2.801$, $\eta^2 = 0.002$, $P = 0.014$). There was also a three-way interaction between sex, restriction type and week (three-way ANOVA, $F_{(5.571,200.546)} = 3.146$, $\eta^2 = 0.002$, $P = 0.007$; Fig. 4). A main effect of week was observed (three-way ANOVA, $F_{(5.571,200.546)} = 36.327$, $\eta^2 = 0.029$, $P = 0.001$).

Mice on a HFHS diet are less motivated to perform FR and PR tasks for reward than mice on a standard chow diet

In the water manipulation groups, mice on a HFHS diet (condition C) required more sessions to reach advancement criteria on the FR task than mice on standard chow diet (condition B) (two-way ANOVA, $F_{(1,51)} = 7.412$, $\eta^2 = 0.119$, $P = 0.009$; Fig. 5a). Sessions to reach criterion were not significantly affected by sex (two-way ANOVA, $P = 0.31$) and there was no sex-by-diet interaction (two-way ANOVA, $P = 0.105$; Fig. 5a). When comparing the rate of responses across FR reward schedules and diet groups, as the number of responses required to obtain reward increased, the rate of response also increased (three-way ANOVA, $F_{(2.107,107.482)} = 123.241$, $\eta^2 = 0.448$, $P < 0.001$; Fig. 5b). However, HFHS mice had overall lower response rates in FR compared with standard-fed mice (three-way ANOVA, $F_{(1,51)} = 7.648$, $\eta^2 = 0.040$, $P = 0.008$; Fig. 5b) particularly during FR5 (Holm post hoc, Cohen's $d = 1.125$, $P < 0.001$; three-way ANOVA, reward schedule-by-diet interaction, $F_{(2.107,\ 107.482)} = 5.187$, $\eta^2 = 0.019$, $P = 0.006$; Fig. 5b). Males on an HFHS diet responded at a lower rate than male mice on a standard chow diet (Holm post hoc, Cohen's $d = 1.074$, $P = 0.004$; three-way ANOVA, sex-by-diet interaction, $F_{(1,\ 51)} = 5.603$, $\eta^2 = 0.030$, $P = 0.022$; Fig. 5b). Notably, female mice did not vary by diet (Holm post hoc, $P = 0.778$), suggesting that the effects of diet observed are driven by the male mice. The main effect of sex was not significant (three-way ANOVA, $P = 0.988$). Overall, mice on an HFHS diet took longer to progress through the FR task and responded at lower rates than standard diet-fed mice, particularly in male mice and when greater responses for reward were required. This finding demonstrates that diet affects training speed and response rates across the FR task.

Following the FR task, mice were run on the PR task. While on 1.5% CA, chow-fed mice had higher breakpoints than HFHS mice (three-way ANOVA, main effect of diet, $F_{(1,51)} = 22.204$, $\eta^2 = 0.245$, $P < 0.001$; Fig. 5c, left), with no effect of sex (three-way ANOVA, $P = 0.914$). Mice on both diets had lower breakpoints at PR4 than at PR8 or PR12 (three-way ANOVA, $P_s < 0.016$; main effect of reward schedule, $F_{(1.660,\ 84.652)} = 9.259$, $\eta^2 = 0.025$, $P < 0.001$; Fig. 5c, left). Number of blank touches differed by diet (three-way ANOVA, $F_{(1,51)} = 11.162$, $\eta^2 = 0.141$, $P = 0.002$; Fig. 5d, left), with no effect of sex. A main effect of reward contingency was observed (three-way ANOVA, $F_{(2,\ 102)} = 11.942$, $\eta^2 = 0.033$, $P < 0.001$; Fig. 5d, left), where blank touches decreased from PR4 to PR8 to PR12.

After returning to 2% CA water, and similar to performance on 1.5%, a main effect of diet on breakpoint was present (three-way ANOVA, $F_{(1,\ 50)} = 4.422$, $\eta^2 = 0.063$, $P = 0.041$; Fig. 5c, right) with standard-chow mice responding more for reward than HFHS-diet mice. Once again, there was also a main effect of PR reward schedule (three-way ANOVA, $F_{(2,\ 100)} = 4.495$, $\eta^2 = 0.010$, $P = 0.014$; Fig. 5c, right) but no main effect of sex (three-way ANOVA, $P = 0.119$). A diet-by-sex interaction was present (three-way ANOVA, $F_{(1,\ 50)} = 4.117$, $\eta^2 = 0.059$, $P = 0.048$; Fig. 5c, right), with a significant drop in performance in HFHS-fed male mice compared with standard chow-fed male mice (Holm post hoc, Cohen's $d = 1.054$, $P = 0.031$; Fig. 5c, right) but no differences observed in female mice (Holm post hoc, $P_s > 0.060$). Blank touches differed across PR reward schedule

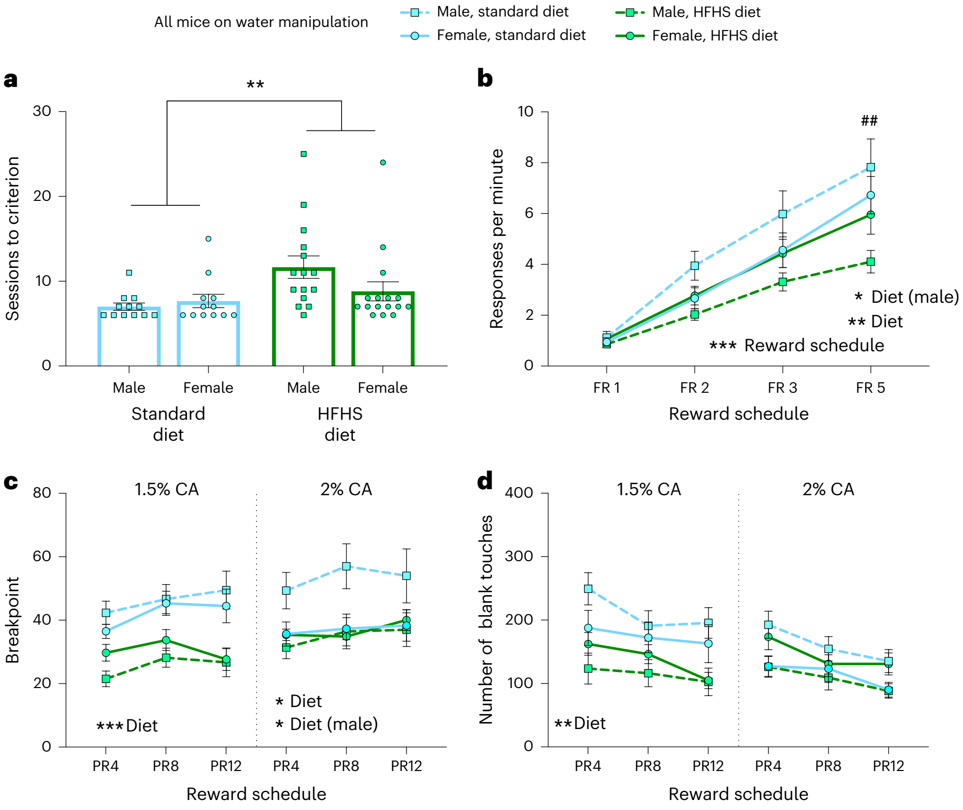

Fig. 5 | HFHS-fed mice are less motivated than standard chow-fed mice in FR and PR tasks. Mice were trained on FR before advancing to PR testing. **a**, HFHS diet-fed mice required significantly more sessions to reach the criterion for advancement to PR. **b**, HFHS diet-fed mice made significantly fewer responses per minute than standard diet-fed mice. This effect was driven predominantly by the males. Overall, mice made more responses per minute with increasing number of responses require to obtain reward. **c**, HFHS diet-fed mice had lower breakpoints than standard diet-fed mice both on 1.5% CA water and 2% CA water. During the second run (2% CA water), this main effect of diet was driven by differences in the male mice. **d**, When on 1.5% CA water, HFHS diet-fed mice made fewer blank touches than standard diet-fed mice, particularly the males. Standard chow diet ($n = 12$ per sex), HFHS diet ($n = 15$ male, $n = 16$ female). Data are presented as mean ± s.e.m.; P values for main effects of two-way mixed model ANOVA (**a**): $^{**}P < 0.01$; P values for main effects and interactions of three-way mixed model ANOVA (**b**–**d**): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; P values for Holm post hoc analyses: $^{\#\#}P < 0.01$ for diet comparison.

(three-way ANOVA, $F_{(2,100)} = 13.431$, $\eta^2 = 0.064$, $P < 0.001$; Fig. 5d, right), with no further main effects (three-way ANOVA, $P_s > 0.467$). Finally, there was a sex-by-diet interaction for blank touches (three-way ANOVA, $F_{(1,50)} = 7.727$, $\eta^2 = 0.091$, $P = 0.008$; Fig. 5d, right) with a trend for fewer blank touches in male mice on an HFHS diet than male mice on a standard chow diet; however, this (Holm post hoc, $P = 0.098$) and other pairwise comparisons did not reach statistical significance (Holm post hoc, all others, $P_s > 0.222$). It is possible that the observed male-biased decreases in PR performance are driven, at least in part, by larger physiological effects of an HFHS diet compared with females.

Collectively, these results demonstrate that water-manipulated mice fed an HFHS diet were less motivated to obtain reward than chow-fed mice in touchscreen PR. Male mice on an HFHS diet were impaired compared with their chow-fed counterparts.

Water-manipulated mice consuming an HFHS diet perform similarly to standard-chow mice in the PVD

Following PR, water-manipulated mice performed the PVD task. All mice improved in performance across the nine sessions (three-way ANOVA, $F_{(5.783,289.169)} = 70.476$, $\eta^2 = 0.458$, $P < 0.001$; Fig. 6a), with no significant effects of sex, diet or any interactions observed (three-way ANOVA, $P_s > 0.065$). Overall, mice performed fewer correction trials across sessions (three-way ANOVA, $F_{(4.911,245.567)} = 79.626$, $\eta^2 = 0.521$, $P < 0.001$; Fig. 6b). HFHS-diet mice made fewer correction trials than standard fed mice (three-way ANOVA, main effect of diet, $F_{(1,50)} = 4.145$, $\eta^2 = 0.009$, $P = 0.047$; Fig. 6b), but there was no effect of or interaction with sex or sessions (three-way ANOVA, $P_s > 0.057$). Lastly, PI decreased across sessions (three-way ANOVA, $F_{(4.521,226.062)} = 21.108$, $\eta^2 = 0.242$, $P < 0.001$; Fig. 6c) but did not differ between diet groups or sex (three-way ANOVA, $P_s > 0.162$), and no significant interactions were observed (three-way ANOVA, $P_s > 0.203$).

HFHS diet induces significant weight gain in water-manipulated mice with few other health concerns

Mice were monitored for health using a scoring rubric for categories of posture and grooming, activity levels, signs of dehydration and eating and drinking (Table 1; Methods). Low scores indicated no substantial health changes within a single category (<2 out of 3) or across categories (<5 out of 11). All standard-chow, water-manipulated mice reported scores of 1 or less in activity levels or sign of dehydration, indicating no major health concerns. Only four (two male, two female) HFHS-diet mice were reported to have cumulative scores of either 2 or 3 within the activity level or posture categories that prompted monitoring by veterinary care. These scores later decreased as mice acclimatized to the combination of CA water manipulation and an HFHS diet.

Body weight was monitored throughout the experiment (Fig. 7). Overall, HFHS diet-fed mice weighed significantly more than standard diet-fed mice (three-way ANOVA, main effect of diet, $F_{(1,51)} = 42.98$, $\eta^2 = 0.149$, $P < 0.001$) and male mice were heavier than female mice (three-way ANOVA, main effect of sex, $F_{(1,51)} = 162.695$, $\eta^2 = 0.565$, $P < 0.001$). There was a significant sex-by-restriction type interaction (three-way ANOVA, $F_{(1,51)} = 7.755$, $\eta^2 = 0.027$, $P = 0.008$), revealing that the effect of diet was greater in male mice compared with female mice but significant in both (Holm post hocs, males, Cohen's d = 2.318,

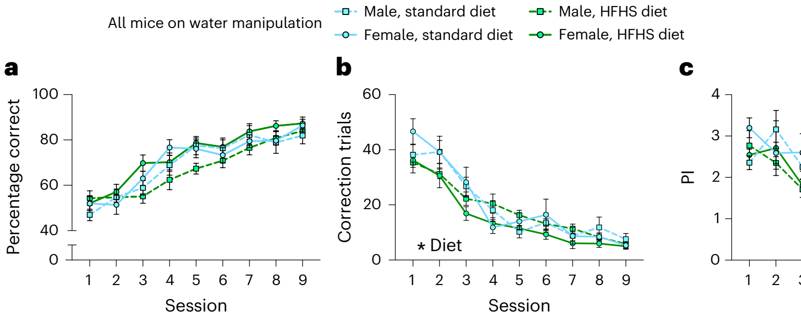

Fig. 6 | Diet does not affect percentage correct responding, but mice fed an HFHS diet required fewer correction trials during PVD learning. **a**, Neither sex nor diet affected percentage correct responding across the nine sessions of PVD. **b**, Mice fed an HFHS diet required more correction trials than standard diet-fed mice during PVD. There was no interaction between sex and diet. **c**, Perseverative responding was not affected by diet or sex. Standard chow diet ($n = 12$ per sex), HFHS diet ($n = 15$ per sex). Data are presented as mean ± s.e.m., P values for main effects of three-way mixed model ANOVA: $^*P < 0.05$.

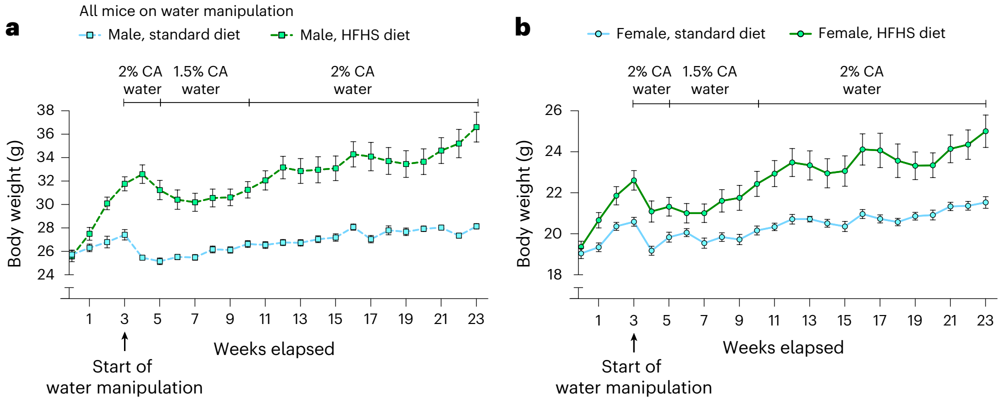

Fig. 7 | Average weekly body weight of mice on water manipulation. **a**,**b**, Mice were fed standard chow or an HFHS diet ad libitum from week 0 (baseline) until the end of the experiment. Mice were weighed three times per week. Starting at week 3, mice received 2% CA water (standard diet: $n = 12$ per sex, HFHS diet: $n = 16$ per sex). Mice began consuming 2% CA water on week 4 but were reduced to 1.5% CA water from week 5 to 8 to combat rapid body weight loss. Following weight stabilization at week 8, mice were returned to 2% CA water until the end of the study. Following week 3, mice were weighed and had their health monitored six times per week. Data are separated by sex: male data (**a**) and female data (**b**). Data are presented as mean ± s.e.m.

$P < 0.001$; females, Cohen's $d = 0.936$, $P = 0.011$). As weeks elapsed, mice on an HFHS diet gained more weight compared with mice on a standard diet (three-way ANOVA, week-by-diet interaction: $F_{(3.242,\ 165.325)} = 7.083$, $\eta 2 = 0.005$, $P < 0.001$) once started on CA water manipulation. Male mice gained more weight than female mice (three-way ANOVA, week-by-sex interaction, $F_{(3.242,\ 165.325)} = 3.121$, $\eta^2 = 0.002$, $P = 0.024$; no significant three-way sex-by-diet-by-week interaction: $P = 0.132$; Fig. 7).

Discussion

Specialized diets, such as demyelinating and HFHS diets, are used as experimental manipulations in many studies but their use in food-motivated tasks is problematic owing to the need in such tasks for adequate motivation for food reward. The present findings show that water manipulation using CA water supports behavioral performance on strawberry milkshake-rewarded touchscreen-based tasks in both standard-fed and HFHS-fed mice. Notably, differences in touchscreen performance were observed between diet groups in the PR task, which is highly sensitive to changes in motivation, but this decrease in motivation was not sufficient to affect performance on the PVD task used to assess visual discrimination learning. Thus, CA water manipulation allows testing of mice on diets such as HFHS on appetitively motivated touchscreen tests of cognition.

Water manipulation is sufficient to motivate standard chow-fed mice on touchscreen tasks (comparison 1)

Previous work by Urai et al.[24] and Reinagel[25] confirmed the efficacy of CA water manipulation as a motivator in an apparatus that paired a computer display with either a steering wheel or licking ports for responses, respectively. In line with those publications, the present study also found CA water manipulation to be sufficient to elicit behavioral performance on touchscreen tests of motivation and visual discrimination. Under standard diet conditions, both CA water manipulation and food restriction were equally effective in motivating standard chow-fed mice to respond for strawberry milkshake reward without changes in latency to respond (Supplementary Fig. 1) on a touchscreen-based motivation task. This motivated performance further extended to learning in a PVD task on which there were no differences between water-manipulated and food-restricted mice. Thus, CA water manipulation is a sufficient motivator of behavior in touchscreen chambers in standard-fed mice, extending the utility of water manipulation as a motivator for behavioral tasks in animal research.

HFHS diet reduces motivation in the PR task but does not restrict performance in PVD learning motivated by CA water manipulation (comparison 2)

As the need for food restriction was previously recommended for touchscreen testing, rendering the use of specialized diets in this testing modality problematic, we sought to determine whether CA water manipulation—which enables animals to both drink and eat ad libitum—is sufficient to drive responding in touchscreens when mice were fed an HFHS diet, which is perhaps the most satiating of all specialized diets. It is documented that an HFHS diet typically results in impaired motivation[29,30]. Particularly, it is both the composition of the food, such as increased sugar and fats, and caloric intake that affect motivation[31,32]. An HFHS diet often leads

Table 2 | Risk–benefit evaluation of motivational protocols in mice (food restriction, CA water manipulation and water restriction)

	Food restriction[12,22,43–45]	CA water manipulation	Water restriction[12,22,43–45]
Weight loss (<15%) events	Frequent; week 1–2 (design targets 85% body weight)	Rare; week 1; occurred in isolated cases during first week and then weight regained	Frequent if not carefully managed; week 1–2
Weight recovery to ≥85%	Within week 1–2	Within week 1–2	Week 1; efficient recovery period
Health scoring events (as presented in Table 1)	Mild; week 1; some signs of stress or discomfort if protocol followed; lethargy or reduced daily activity especially if mishandled	Mild; week 1–3; reduced grooming/posture, urine/feces production and reduced activity during early phase	Moderate; week 1–2; lethargy or reduced daily activity especially if mishandled
Peak vulnerability period	Week 1–2 of implementation	Week 1; especially during initial adaptation	Week 1–2; especially during initial adaptation
Dehydration	Low risk	Low risk; managed with CA concentration adjustments or periodical access to tap water	High risk after 24 h: skin tenting, hypodipsia, anorexia if protocol deviates
Health benefits	Fewer age-related diseases; longer life expectancy	Improved gut and liver health	Unknown
Diet effects	No major complications	No major complications, permits weight gain from baseline	No major complications, reduced overall diet intake
Animal intervention needs	Moderate; caloric supplement, increased food amount or soft chow as needed	Minimal; occasional switch to 1% CA or tap water within week 1 or as needed	Moderate; requires rehydration (hydrogel or water access); water access over weekends
Ease of implementation	Easy to implement; requires daily feeding and weight monitoring (3× per week to daily)	Easy to implement; weekly water change and daily weighing; requires daily health check	Requires strict timing and compliance protocols; requires daily health check
Operant test performance (with sex considerations)	High motivation and learning; consistent performance between male and female mice	Consistent performance on learning tasks between male and female mice; lowered motivation with weight gain, particularly in male mice	Effective especially with fluid rewards; consistent performance on motivation and learning tasks, including between male and female mice
Overall risk classification	Moderate (known, predictable risks)	Moderate (rare interventions and stable outcomes)	Moderate (higher monitoring)
Refinement potential	Appropriate for short-term studies in adolescent mice or long-term studies in adolescent mice when weight is maintained relative to the mean ad libitum BW of the animal strain; appropriate for any duration in adult animals	Recommended for adolescent animals, diet studies or drug administration through diet	Suitable for longer-duration studies
Risk–benefit summary	Provides robust motivation and well validated in animal behavior; potential impact on growth and weight stability in developing animals; rare interventions required	Minimal severity, high reproducibility and ease of use, making it suitable for long-term, adolescent or diet-based studies. Rare interventions required (for example, diluted CA for 1 h); easy to implement	Effective to elicit behavior, requires extensive monitoring and strict protocol adherence, may be best when liquid reward-based paradigms necessitate its use

to metabolic dysfunction, dysregulated dopamine signaling[18,19], reduced reward sensitivity[19,32] and lowered activity levels[33], all of which would be expected to substantially demotivate animals and/or reduce task engagement. Even with these well-known demotivating effects and reductions in task engagement, we observed that CA water manipulation was sufficient to motivate mice on an HFHS diet to complete the PR task, a task highly sensitive to changes in motivation. While HFHS-fed mice on water manipulation took longer to train on the task and showed decreased motivation compared with ad libitum standard chow-fed mice (also motivated with CA water manipulation), they could perform the task (without changes in latency to respond; Supplementary Fig. 2). Consequently, we were able to robustly quantify differences in motivation for an appetitive reward in mice on an HFHS diet versus a standard-chow diet.

This study investigates the effects of an HFHS diet on discrimination learning as measured by the PVD task, which may be due to limitations in restriction protocols to motivate behavior. Rather, most studies observe HFHS diet-induced impairments in tests of spatial memory, working memory and object recognition that depend on spontaneous, nonrewarded testing paradigms in rodents[34] and humans[31,32,35]. While an HFHS diet led to lower breakpoints on a motivation-sensitive PR task, this did not translate into learning impairments on the PVD task. This has similarly been seen in rats that were food restricted on a standard-chow diet and provided 70 ml per day of high sucrose water on the same PVD task[36]. The PVD task, similar to the FR1 task, requires only a single response to the correct stimulus to obtain a reward, leading to consistent reinforcement with each correct response. This simple response requirement probably protected performance from motivational deficits observed in tasks with higher-effort demands such as PR, where multiple responses revealed motivational impairments. Thus, it is possible that CA water manipulation will similarly elicit performance in touchscreen tasks that assess other cognitive domains, such as attention, even in mice on specialized diets. In this study, we were able to conclude that an HFHS diet does not impact the rate of learning on a PVD task compared with a standard diet.

Sex-specific differences between restriction types and diets

Beyond the expected and normal differences in body weight between male and female mice, there were notable sex differences observed in this study, especially following HFHS-diet consumption. In comparison 1 (standard-diet comparison), sex differences were observed in PR and PVD task performance. During PR, male mice exhibited higher breakpoints on PR8 compared with PR4 and P12, irrespective of restriction type. Early reports found that in standard-fed, food-restricted animals, male and female mice achieve similar reinforcer counts on PR tasks[37], although females may show greater effort under higher FR requirements[38]. Interestingly, in PVD, males made more errors (correction trials) and perseverated on those errors to a greater extent than females did. However, this difference did not lead to differences in overall task accuracy. Supporting this pattern, Chen et al.[39] reported that female mice outperformed males in a touchscreen-based visual decisionmaking task, acquiring stimulus–reward associations more rapidly, possibly owing to differences in learning strategy. In that study, male mice used more inconsistent, experience-dependent strategies, which may help explain the higher number of errors observed in males in the present study[39].

In comparison 2 (water-manipulation comparison), males on an HFHS diet exhibited lower response rates during the FR task, showed reduced breakpoints in the PR task and tended to make fewer nonspecific responses when compared with males on a standard-chow diet. This aligns with findings from Ibias et al.[28], where male mice on an 8-week high-fat diet showed reduced lever pressing for palatable food, suggesting diet-induced decreases in motivation. Females showed no differences in weight between restriction types and a more moderate increase in weight on an HFHS diet when compared with the substantial weight gains seen in HFHS-fed males. Most studies examining diet and PR performance either restrict analyses to male animals or combine diet manipulation with food restriction, which results in inconsistent findings potentially owing to an absence of weight gain[40]. Further investigation into how body weight, adiposity and circulating nutrients resulting from different diets (including drinking water) affect motivation and learning in males and females is needed.

CA water manipulation is practical to implement and promotes positive health outcomes in mice

The use of water manipulation resulted in no substantial health changes and permitted mice to maintain or gain weight throughout the study while maintaining task performance. Mice given CA water stabilized or regained their weight and presented few signs of dehydration, lowered activity levels and abnormal posture or grooming. However, the absence of complementary welfare indicators—such as nest-building, burrowing or noninvasive stress measures (for example, fecal corticosterone metabolites)—which would permit a more comprehensive assessment of the impact of water-manipulation protocols on animal well-being in future studies, is a limitation. Water manipulation permitted changes in weight during development and diet consumption in contrast with the food restriction group which, by design, is maintained at 85–90% of baseline weight throughout testing. Therefore, CA water manipulation may be a useful alternative in studies using adolescent mice to permit normal growth and development.

In addition, water manipulation requires minimal experimenter labor when compared with food restriction or other types of water restriction. CA water preparations were completed at the start of each week and animals only required daily monitoring and weighing. Unlike food restriction, there are also no concerns regarding time of feeding (especially relative to behavioral experiments), missed feedings or competition over food in grouped-housed animals. CA may also offer health benefits in animals. Urai et al.[24] highlight that CA increases the acidity of water, which may benefit gut health in animals. Previous literature has also suggested that CA is an antioxidant and limits excessive generation of reactive oxygen species and free radicals, which benefits liver health[41].

Considerations for future use

Water manipulation affected neither motivation nor learning in standard chow-fed mice. By contrast, an HFHS diet reduced motivation in water-manipulated mice compared with standard-fed mice on water manipulation. Therefore, it is critical for researchers to consider their control groups and confounds that may exist with future use of water manipulation. If diet is the dependent variable in an experiment, it will be important to consider how diet may affect motivation in testing and how that can impact the dependent variables collected. Even in experiments where diet is consistent across experimental groups, caution should be used to ensure accurate interpretation of the data, including how the results may generalize to standard and other nonstandard diets.

Notably, this study was conducted within a single research facility and by a single team of experimenters, which may limit the generalizability of findings. However, touchscreen-based cognitive tasks have demonstrated strong reproducibility across independent laboratories and cohorts[6,7,42], supporting the broader applicability of the present findings. While interlaboratory validation was beyond the scope of this work, future studies replicating this CA water-manipulation protocol across different sites would bolster the reliability and robustness of this method.

Both water manipulation and food restriction are viable and effective strategies for motivating behavioral performance in rodents, with each offering distinct advantages depending on the intended research questions. Water manipulation provides a lower-severity alternative to food restriction that permits normal growth and is well suited for long-term, adolescent or diet-manipulation studies. By contrast, food restriction delivers robust motivational enhancement in operant tasks and remains a gold standard in many behavioral paradigms. The choice between these methods should be guided by the specific demands of the tasks, age and health status of the animals and the intended cognitive or metabolic outcomes. Table 2 presents the health, performance and welfare considerations of food restriction, water manipulation and water restriction, allowing for context-sensitive risk–benefit evaluations. In addition, Supplementary Figs. 3–6 show the main measures of this study again with individual data points represented to give an estimate of the spread and variability of individual-level data. Future studies incorporating noninvasive welfare metrics (for example, nest building and fecal corticosterone) will help further refine the selection of motivational protocols across research domains.

Conclusion

Water manipulation using CA water is an effective alternative to food restriction in motivating mice across both standard and HFHS diet conditions. While motivation was reduced in mice on CA water when consuming an HFHS diet, mice were still able to perform the tasks. Importantly, these motivational changes did not impact learning performance, highlighting the potential utility of water manipulation using CA in future studies where food restriction may not be feasible or desirable.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41684-026-01711-y.

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Methods

All experiments were conducted in compliance with the standards set by the Canadian Council of Animal Care and under direct veterinary supervision at the Western University (animal utilization protocol 2021-082).

Experimental design

A total of 72 C57BL/6J mice (36 males and 36 females, Jackson Laboratory, USA) arrived at 8–12 weeks of age. Mice were group-housed by sex with four mice per cage in a room with controlled temperature (23 ± 1 °C) and humidity (50 ± 1%) under a reverse 12 h light–dark cycle (lights off at 09:00). Animals were housed in a shoebox cage provisioned with Biofresh bedding, Envirodri and nestlets for nesting, as well as twist bits, diamond twists, wooden chew sticks and a cardboard tunnel to provide environmental enrichment. The complete study design is shown in Fig. 1.

Mice were acclimated to their cages for 5 days with ad libitum access to standard Teklad diet (Envigo) and untreated water. Mice were each weighed and the average body weight per cage was calculated. Cages were then randomly assigned in order of cage barcode (used only for billing) to standard preweighed diet (Bioserv F0078, 3.6 kcal/g, 5.6% fat, 59.1% carbohydrates, 18% protein; $n = 20$ per sex) or HFHS chow (Bioserv F6724, 4.57 kcal/g, 21.2% fat, 48.5% carbohydrates, 17.3% protein; $n = 16$ per sex). Stratified randomization based on starting weight was used to ensure comparable baseline characteristics across diet groups. Mice were maintained on their respective diets for 3 weeks, after which baseline weights were calculated as mean body weight over 3 days. Following baseline calculations, food restriction and water-manipulation protocols were implemented and randomly assigned by order of cage barcode, stratified by sex. In total, 16 mice ($n = 8$ per sex) in the standard-diet group were randomly assigned to undergo food restriction to 85–90% of their baseline weight. A total of 24 mice ($n = 12$ per sex) received an ad libitum standard diet, and 32 mice ($n = 16$ per sex) received an ad libitum HFHS diet (Fig. 1). All mice with ad libitum access to diet also received 2% CA water manipulation ad libitum. In total, 2 g CA (CA anhydrous, Thermofisher Scientific) was dissolved in 100 ml tap water to produce 2% CA water.

Mice were weighed 3–6 times per week in accordance with the animal use protocol. Mice undergoing food restriction had a healthy weight defined as 85–90% of their baseline weight, whereas mice consuming 2% CA water had no upper boundary of permissible body weight. The 2% CA water-manipulated mice had their weight recorded between 10:00 and 12:00 each day and were administered CA water with a reduced concentration of CA if they experienced abrupt weight loss to an amount less than 85% of their baseline weight. Food-restricted mice found to be underweight were provided additional diet (minimum of 0.5 g increase); water-manipulated mice found to be underweight were provided 1 h of access to 0.5% CA water to encourage increased water consumption. Health scores were provided for each mouse on the basis of their posture and grooming, dehydration levels, activity and eating or drinking using a health scoring rubric (Table 1). All mice were included in weight measures and health scoring even if removed from touchscreen testing.

Touchscreen behavioral testing

All touchscreen behavioral testing was completed using standard Bussey–Saksida mouse touchscreen chambers (model 80614, Lafayette Instrument Company) as described in detail elsewhere[7] and using task specific standard operating procedures published to https://touchscreencognition.org. In brief, the apparatus contains a trapezoidal chamber with a touchscreen at one end and a reward magazine on the other end. A space in front of the touchscreen permits the insertion of removable masks (black plastic sheets with apertures that allow access to the touchscreen) specialized to each cognitive test. The reward magazine illuminates and dispenses liquid milkshake reward (Nielson's Strawberry Milkshake) upon successful trial completion following interaction with the touchscreen. Therefore, the number of trials completed is equivalent to the number of rewards obtained. Mice were habituated to the chambers and milkshake rewards over 3 days and were given several pretraining stages in order that the mice learned to interact with the touchscreens and successfully retrieve the reward[9,10,46].

Touchscreen tests of motivation (FR and PR tasks)

Following habituation and pretraining, mice were trained for PR using the FR task. The FR task comprised several reward schedules, with each reward schedule requiring a different number of nose pokes to the stimulus (white square) to obtain a reward. For example, mice were trained to collect a reward (~24 µl dispensed via 800 ms pulse of a peristaltic pump) by completing one touchscreen response to the stimulus in the FR1 reward schedule[9]. Following each reward collection, there was a 20-s intertrial interval (ITI) before the next trial could be initiated and for a stimulus (white square) to appear in the center of a five-window mask. After achieving 30 rewards in 60 min, mice were advanced to the subsequent reward schedule[9]. The following reward schedules FR2, FR3 and FR5 required 2, 3 or 5 responses to the stimulus, respectively[46]. Therefore, FR5 required the greatest effort, requiring five responses to the stimulus for a single reward. The number of responses, time of session completion and rewards collected were recorded. To examine the rate of responding, the number of responses per minute were calculated. In addition, the number of sessions to complete all the FRs was totaled.

Following successful completion of FR5, the PR task began. One water-manipulated male HFHS-diet mouse failed to reach the criterion and therefore did not progress from FR to PR. One water-manipulated female HFHS-diet mouse was removed following the first round of PR owing to health complications not related to diet or CA water. PR task parameters were identical to those in the FR task with the exception that the number of responses to the touchscreen required to obtain a reward differed: the number of touches required increased incrementally within a session, such that the responses required for reward in PR4 increases by four for each subsequent reward (that is, trial 1 required one response; trial 2, five responses; trial 3, nine responses, and so on). After completing PR4, mice completed PR8 and PR12, where the number of required responses for a reward increased by 8 or 12 on each trial, respectively. Mice were only given one PR session (either PR4, PR8 or PR12) per day. To examine motivation, the breakpoint for each mouse was calculated; breakpoint is the number of responses made in the final successfully completed trial. For example, in PR4, if a mouse successfully completed the first four trials with reward requirements of 1, 5, 9 and 13 responses, respectively, but failed to complete the next (17 responses for reward), it thus reached a breakpoint of 13. In addition, reward latency and the number of nonstimulus touches (blank touches) were recorded.

PVD task

Following FR/PR, mice performed the PVD task[7]. Mice were first pretrained for PVD on punish incorrect, where correct touches were rewarded and blank touches triggered a 5-s timeout with the house light illuminated. Mice needed to complete ≥24/30 trials correctly (that is, a minimum of 24 rewards) within 60 min for two consecutive sessions before moving on to PVD. One water-manipulated male HFHS-diet mouse failed to reach the criterion during pretraining and did not move on to PVD. The PVD task required mice to discriminate between two images (diagonal lines 45° clockwise or anticlockwise from vertical) and respond to the correct (rewarded) stimulus image (S^+) and withhold responding to the incorrect (unrewarded) stimulus image (S^-) when they were both displayed simultaneously on either side of the touchscreen[7]. The relative left and right presentation of the stimuli varied pseudo-randomly across each trial, and stimuli were not displayed in the same location for more than three consecutive trials. By contrast, an incorrect response to the S^- resulted in a 5-s illumination of the chamber by the house light. Following the 5-s penalty, a 20-s ITI was initiated. Following the ITI, a correction trial began in which the stimuli were presented in the same configuration as the previous trial and this trial configuration was repeated until the mouse selected the S^+ and collected a reward. Correction trials were quantified but were not included in the session trial limit (30) nor in the session accuracy score. Mice completed a total of 9 sessions of 30 successful trials each. The percentage correct responses out of 30 trials (that is, accuracy) and the number of correction trials were recorded for each session. When a mouse did not reach 30 trials in a single session, it was tested the next day on the trials remaining to reach 30 trials; therefore, each session analyzed consisted of a total of 30 trials. Owing to running errors, one mouse (male, HFHS diet, water manipulated) completed 33 trials during session 1 and another (male, HFHS diet, water manipulated) completed only 20 trials during session 3. Formulae for PIs were adapted in these cases.

Data analysis

The dependent variables were examined using two separate analyses to address the main questions of this study: (1) comparison of the restriction protocol (food restriction, CA water manipulation) and sex (male, female) within the standard diet groups (that is, mice given standard chow) and (2) a comparison of diet (standard, HFHS) and sex (male, female) within water-manipulated groups (that is, mice given ad libitum CA water). The comparison between mice on a standard diet with food restriction and those on an HFHS with water manipulation was considered irrelevant to the research aims; therefore, these conditions were not directly compared statistically, which reduced potential type 2 errors. Moreover, using the same standard-diet mice consuming CA water in both analyses align with the principles of the 3Rs of animal research, reducing animal use while maximizing data collection from the same cohort. This design addresses (1) whether CA water and food restriction could sufficiently motivate mice consuming standard chow to a similar degree during touchscreen testing and (2) whether CA water can motivate mice consuming an HFHS diet to a similar degree as standard chow-diet mice.

Sample size was determined a priori using G*Power 3.1 for a one-way ANOVA. On the basis of an effect size of $f = 0.4$ (partial $\eta^2 \approx 0.16$), $\alpha = 0.05$ and power $(1 - \beta) = 0.80$, an estimated total of 48 animals (12 per group) was calculated to be sufficient to detect group differences. While 8–10 animals per group is common in touchscreen behavioral studies, a larger sample size ($n = 16$–24 per experimental group and $n = 8$–12 per sex) was used to account for potential attrition, training failure or health-related exclusion and to ensure adequate power for subgroup comparisons involving diet, water manipulation and sex.

Full blinding during the experiment was not feasible owing to the visible differences in diet texture, color and odor as well as the need to ensure that animals were provided their allocated water condition. Cages were labeled with diet and water type to ensure proper care and health monitoring. All touchscreen tasks were fully automated, with data recorded and scored by the system, requiring no manual experimenter input during outcome assessment.

Data were analyzed using JASP (Version 0.16.3, Intel). Three-way mixed model ANOVAs were used, with restriction protocol (food restriction, CA water manipulation) or diet (standard chow, HFHS) and sex (male, female) as between-subject factors and day, week or session as the within-subject repeated factors. In instances where sphericity assumptions were violated (Mauchly's test), Greenhouse–Geisser corrections were applied. When an interaction effect was detected, Holm post hoc analyses were performed where appropriate. Two-way ANOVAs were run on sessions to criterion data, with restriction protocol (food restriction, CA water manipulation) or diet (standard chow, HFHS) and sex (male, female) as between-subject factors. Data are presented as mean ± standard error of the mean (s.e.m.) in the main figures of the text, but figures showing mean ± standard deviation (s.d.) and individual data points are provided in Supplementary Figs. 3–6. Significance was set at $\alpha < 0.05$.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Datasets from these studies are available on MouseBytes.ca at https://doi.org/10.58064/gwez-2h31.

References

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Author contributions

L.D., O.R.G.-S., J.R.D., T.J.B. and L.M.S. conceptualized the study. L.D., O.R.G.-S., J.A. and J.R.D. performed the behavioral analyses. L.D., O.R.G.-S. and P.A.S.S. processed the data. L.D., O.R.G.-S., P.A.S.S. and T.J.B. wrote, reviewed and edited the paper. J.R.D., P.A.S.S., T.J.B. and L.M.S. supervised the project. T.J.B. and L.M.S. coordinated project administration and secured funding. All authors have read and agreed to the published version of the paper.

Competing interests

T.J.B. and L.M.S. have established a series of targeted cognitive tests for animals, administered via touchscreen within a custom environment known as the 'Bussey–Saksida touchscreen chamber'. Cambridge Enterprise, the technology transfer office of the University of Cambridge, supported commercialization of the Bussey–Saksida chamber, culminating in a license to Campden Instruments. Any financial compensation received from commercialization of the technology is fully invested in further touchscreen development and/or maintenance. Campden Instruments play no role in the conceptualization, design, data collection, analysis, decision to publish or preparation of the manuscript. The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41684-026-01711-y.

Correspondence and requests for materials should be addressed to Paul A. S. Sheppard or Lisa M. Saksida.

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Give P values as exact values whenever suitable.

☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ ☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on [statistics for biologists]() contains articles on many of the points above.

Software and code

Policy information about [availability of computer code]()

Data collection	No previously unreported custom computer code or algorithm used in data collection. Data were collected using ABET II software from Lafayette.
Data analysis	No previously unreported custom computer code or algorithm used in data analysis. Data were analyzed in JASP (0.16.3 Intel).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined based on the required sample size to produce significant power in previous touchscreen behavioural experiments
Data exclusions	Data from individual mice were removed from analyses only when health concerns arose or when the mouse did not reach performance criteria for a specific task.
Replication	All SOPs and procedures used in this study are publicly available. Further, the use of touchscreen testing increases the ease of replicating experiments using identical parameters. Attempts at replicating the data within the study were not performed.
Randomization	Experimental groups were randomly assigned on a cage-level basis (all mice in a cage were assigned the same intervention) such that the required number of mice per sex were in each experimental group.
Blinding	Experimenters were not blind to experimental group during testing. However, data collection was automated by the touchscreen apparatuses. Further, touchscreen testing allows for minimal experimenter involvement in testing, removing sources for bias.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57Bl6/J mice from Jackson Laboratories. Half male, half female. Mice were 2 months of age at the beginning of the experiment
Wild animals	This study did not involve wild animals
Field-collected samples	This study did not involve field-collected samples
Ethics oversight	All experiments were conducted in compliance with the standards set by the Canadian Council of Animal Care and under direct veterinary supervision at the Western University (animal utilization protocol 2021-082).

Note that full information on the approval of the study protocol must also be provided in the manuscript.