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Chronic calorie-dense diet drives differences in motivated food seeking between obesity-prone and resistant mice

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Abstract

Obesity results from overconsumption of energy, partly because of the inability to refrain from highly palatable rewarding foods. Even though palatable food is available to everyone, only a fraction of the population develops obesity. We previously showed that following chronic exposure to highly palatable food animals that gained the most weight also showed addictive-like motivation to seek for palatable food. An important question remains—is this extreme, addictive-like, motivation to consume palatable food the cause or the consequence of diet-induced obesity? Here, we show that obesity-prone (OP) mice exhibit higher motivation for palatable food consumption compared with obesity-resistant mice even before developing obesity, but that the full manifestation of this high motivation to eat is expressed only after chronic exposure to high-fat-high-sugar (HFHS) diet. HFHS diet also impairs performance in the operant food-seeking task selectively in OP mice, an impairment that persists even after 2 weeks of abstinence from HFHS food. Overall, our data suggest that while some aspects of food motivation are high in OP mice already before developing obesity, the chronic exposure to HFHS food accentuates it and drives the development of obesity.

KEYWORDS

food-seeking behavior, motivation, obesity, obesity predisposition

1 | INTRODUCTION

Obesity is a physical pathology that originates in the brain. An increasing proportion of the global population suffers from obesity, and despite intensive research, numbers keep increasing.[1,2] Many factors participate in driving the obesity epidemic in the modern world, primarily overeating of highly palatable calorie-dense food. Curiously, even though highly palatable food is readily available for the entire population, only part of the population develops obesity.

The difference between individuals in gaining weight and developing obesity has led to the notion that some individuals may be more prone to develop obesity than others.[3,4] Studies aiming at understanding the mechanisms that underlie this proneness to obesity have identified various elements (such as abnormality in leptin function,[5] gut microbiota,[6] hypothalamic gene expression,[7,8] and others) that are found in obesity-prone animals and have important roles in metabolic processes, fat storage, and hypothalamic function.

The reported desire of obese people to eat palatable food presents a different aspect for the disease—uncontrolled motivation to eat rewarding, palatable food. Indeed, differences between structures in the reward system of obese and nonobese humans[9] or rodents[10-13] have been described, and it was suggested that obese individuals share behavioral and neurobiological similarities with those addicted to drugs.[14-16] When examining rats after chronic exposure to cocaine, two populations can be identified—those that are prone to develop addiction and those that are resistant[17,18] (some mouse models of addiction also reveal populations that differ in their motivation to obtain the reward,[19,20] but these are not yet established for

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cocaine[21]). In previous studies, others and we found that obesity-prone rats show behavioral and neural properties similar to those of cocaine-exposed rats.[10,13] Thus, the proneness to develop obesity may be strongly affected by motivational processes.

A major question that remains open is whether the proneness to develop diet-induced obesity and express overeating is predetermined or perhaps all animals are "born equal" and the proneness to develop obesity is evoked by the chronic exposure to highly palatable food. To examine this, we exposed mice to a chronic high-fat-high-sugar (HFHS) diet and identified the obesity-prone (OP) and obesity-resistant (OR) mice. We then tested their motivation to obtain palatable food in several operant tasks. For the first time, we also tested here the motivation of the same mice to obtain palatable food *before* exposing them to the HFHS diet and *after* 2 weeks of abstinence from the HFHS food.

2 | MATERIALS AND METHODS

2.1 | Experimental subjects

Experimentally naive C57bl6/J wild-type male mice weighing 23 to 30 g at the start of the experiment were housed individually with nesting/enrichment material made available. A 12-hour light/dark cycle was always maintained, with lights turned off at 8:00 AM. Experimental procedures were conducted during the dark hours and approved by the Authority for Biological and Biomedical Models in the Hebrew University. Mice were given 7 days to acclimate before experimentation began.

2.2 | Model of diet-induced obesity

After acclimation to the animal facility and the reverse light cycle, mice (n = 24) were first placed on a standard chow diet (Teklad Global 2018, 18% kcal fat; total density = 3.1 kcal/g; Harlan Laboratories Inc., Indianapolis, Indiana) for 4 weeks. Then, the feeding regime was changed, and mice were placed on a HFHS diet (D12451, 45% kcal fat; total density = 4.73 kcal/g; Research Diets Inc.) for a period of 10 to 12 weeks. At the end of the HFHS regime, mice were put back on a standard chow diet for two additional weeks. A control group of mice (n = 15) went through the same protocols but without having access to HFHS at any point. Food intake and body weight were determined twice per week (BJ-410C scales, Precisa, Dietikon, Switzerland) throughout the entire experiment.

2.3 | Operant self-administration protocol

All operant protocols were conducted in mouse operant boxes (MedAssociates, Fairfax, VT, USA) containing two levers (active and inactive), a house light, stimulus light and tone, and a food receptacle with infrared beam for detection of head entries (Figure S1 in the supporting information). Boxes were located in sound-attenuating boxes to minimize external noises. During the first month (while still on chow diet), mice were trained on the operant tasks. The operant session, a modified version of those previously designed to identify addiction-vulnerable versus addiction-resilient subjects,[22,23] consisted of alternating reward-available (designated S+, 15 min × 3) and reward-unavailable (designated S−, 5 min × 3) periods that were paired with distinct discriminative stimuli. During S+ periods, lever pressing on the active lever resulted in the dispensing of a 20-mg palatable food pellet (S07379, 45% kcal from fat, total density = 4.6 kcal/g; Bioserv Inc., Frenchtown, NJ) or with regular food pellet if mouse was in the chow group (F0071, 5.6% kcal from fat, total density = 3.6 kcal/g; Bioserv Inc., Frenchtown, NJ). Responding on the active lever during S−, as well as responding on the inactive lever during either S+ or S− period, resulted in no programmed consequence.

Mice began on a fixed ratio (FR) of 1 under only S+ conditions for 60 minutes. After 3 days, the S− period was introduced. Mice experienced FR1 for three further days before the response requirement was increased to FR3 (3 days) and then FR5 (remainder of protocol).

The progressive ratio session was conducted in a single session after FR5 responding had been established (typically after 3 days of FR5). Progressive ratio schedule was 5, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. The progressive ratio breakpoint was taken as the last step completed before a lapse of 1 hour during which no pellets were earned or the last step completed in 6 hours, whichever occurred first. After the training was completed and all the mice were tested, mice were put on HFHS diet (or chow for control) for 10 to 12 weeks. Then, mice were tested again, beginning with FR3 (2 days) and then FR5 (2 days) and PR (1 day). At the end of this batch of tests, the mice that were on HFHS were put back on standard chow diet for two more weeks. By the end of these 2 weeks, mice were tested again in the same order as before (FR3, FR5, PR). During the tests, mice had ad libitum access to food depending on the diet they were on before. Chow control mice were tested only twice—before and after the 10 to 12 weeks of chow. Food was removed from home cages of all mice 10 hours prior to behavioral testing (most of which were during the "light" part of the light/dark cycle).

Two mice did not reach the criteria in the behavioral tasks and were not included in the behavioral analyses. One mouse was excluded in the PR test after the HFHS diet (outlier using the ROUT method (Q = 1%), pressed the active lever 698 times) but included in all other tests.

2.4 | Locomotion

Locomotion was measured before and after the HFHS diet. Mice were put in a 30 × 30 cm open-field box lit by dim LED white light and contained in a sound-attenuating chamber, were let to move freely, and were filmed for 45 minutes. Analysis of the locomotion was performed using Ethovision (Noldus, Wageningen, The Netherlands) and MATLAB R2017a (MathWorks, Natick, MA, USA).

3 | RESULTS

In this study, we used a diet-induced obesity protocol similar to the one we used previously on rats[10] but on C57bl6/J mice. Mice were first trained on several operant tasks, tested, and then put on a HFHS diet for 10 to 12 weeks (Figure 1A). During this period, we monitored mouse body weight and food consumption (mice were single-housed). At the end of the HFHS diet, we noticed that, similar to rats, mice differed significantly in the amount of weight they gained (Figure 1B and 1C). We thus split mice into those that gained the most weight (measured as the percent of weight gain relative to the body weight on the first day of HFHS diet) (top third, OP mice) and those that gained the least weight (bottom third, OR). As shown in Figure 1D, OP mice gained significantly more weight than OR mice and the difference between the groups was significant already after 20 days. Note that the initial weight was not different between groups (Figure S2). Also, the development of body weight during the month preceding the exposure to the HFHS diet did not differ between the groups,

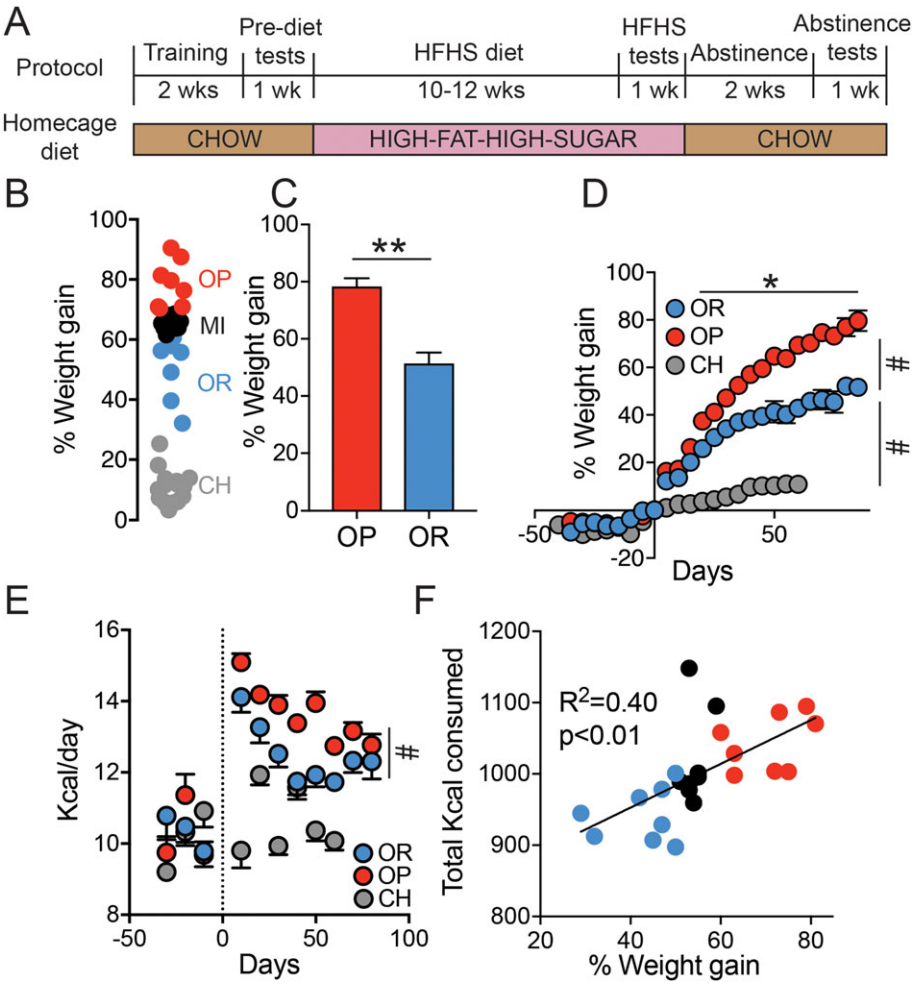

FIGURE 1 Differential effect of a high-fat-high-sugar (HFHS) diet on weight gain in mice. Data presented as average ± SEM. A, Behavioral protocol and dietary regime. All mice were trained and tested ("prediet tests") on the behavioral tasks before their home cage diet was switched to HFHS (day 1). At the end of 10 to 12 weeks of HFHS diet, we tested behavior again ("HFHS tests"). At the end of the tests, we switched the diet back to chow and after 2 weeks of chow tested again the behavior ("abstinence tests"). B, Spread of weight gain (relative to day 1). Top third weight gainers (red) were termed obesity-prone (OP), bottom third (blue) obesity-resistant (OR). Middle (MI) group in black. A control group was fed only on chow (CH, gray). C, OP mice gained significantly more weight than OR mice (78.5 ± 2.8% vs 51.5 ± 3.7%, respectively, two-tailed unpaired *t* test, $t_{(14)} = 5.84$, $^{**}P < 0.01$). D, Time course of weight gain in the OP and OR groups. OP mice gained more weight than OR mice (two-way analysis of variance [ANOVA], main group effect $F_{(1, 307)} = 262.9$, $P < 0.0001$; main time effect $F_{(24, 307)} = 212.3$, $P < 0.0001$; group × time interaction $F_{(24, 307)} = 8.64$, $P < 0.0001$). Values are significantly different already 20 days after the beginning of the HFHS diet (Sidak's multiple comparison test, $t = 3.74$, $P < 0.01$ at 20 days). The control chow group gained significantly less weight than both OP and OR mice. #$P < 0.01$, two-way ANOVA with repeated measures, OP or chow vs OR main group effect. $^{*}P < 0.05$ at day 20 and on, two-way ANOVA multiple comparisons. E, OP mice consumed more kilocalories per day (kcal/day) than OR mice (two-way ANOVA, main group effect $F_{(1, 163)} = 24.93$, $P < 0.0001$; main time effect $F_{(11, 163)} = 29.6$, $P < 0.0001$; group × time interaction $F_{(11, 163)} = 1.853$, $P < 0.05$). The chow group consumed less calories than OR mice (two-way ANOVA, main OR × CHOW effect $F_{(1, 125)} = 8.08$, $P < 0.0001$). #$P < 0.01$, two-way ANOVA with repeated measures, OP vs OR main group effect. F, Correlation between total calories consumed during the HFHS diet and the weight gain

implying for an interaction between genetic predisposition to become obese and the chronic exposure to calorie-dense food. A control group that was fed only on regular chow gained significantly less weight (Figure 1B and 1D) compared with both OP and OR mice.

Weight gain may be explained either by a decrease in mobility (spend less energy), an increase in food intake, or both. Our data show that OP mice consumed more kilocalories (kcal) per day than OR mice (Figure 1E). There was a group × time interaction, indicating that the eating habits changed progressively and in a different manner for each group. As was the case with weight gain, the groups did not differ in energy intake before exposure to the HFHS diet. In addition, a locomotion test revealed no difference in the mobility of OP and OR mice (see Figure 2G-2J). Thus, the enhanced increase in weight in OP mice may be attributed to excessive intake of HFHS food. Indeed, the extent of weight gain is strongly and positively correlated with daily caloric intake (Figure 1F) but not locomotion (not shown). The caloric consumption of a control chow group (10.02 ± 0.4 kcal/day) was lower than that of both OR (11.93 ± 0.3 kcal/day) and OP (13.95 ± 0.3 kcal/day) mice (one-way analysis of variance [ANOVA] on average of days 40-50 of the diet, $F_{(2,\ 28)} = 27.29$, all groups different from each other using Tukey's multiple comparison test.

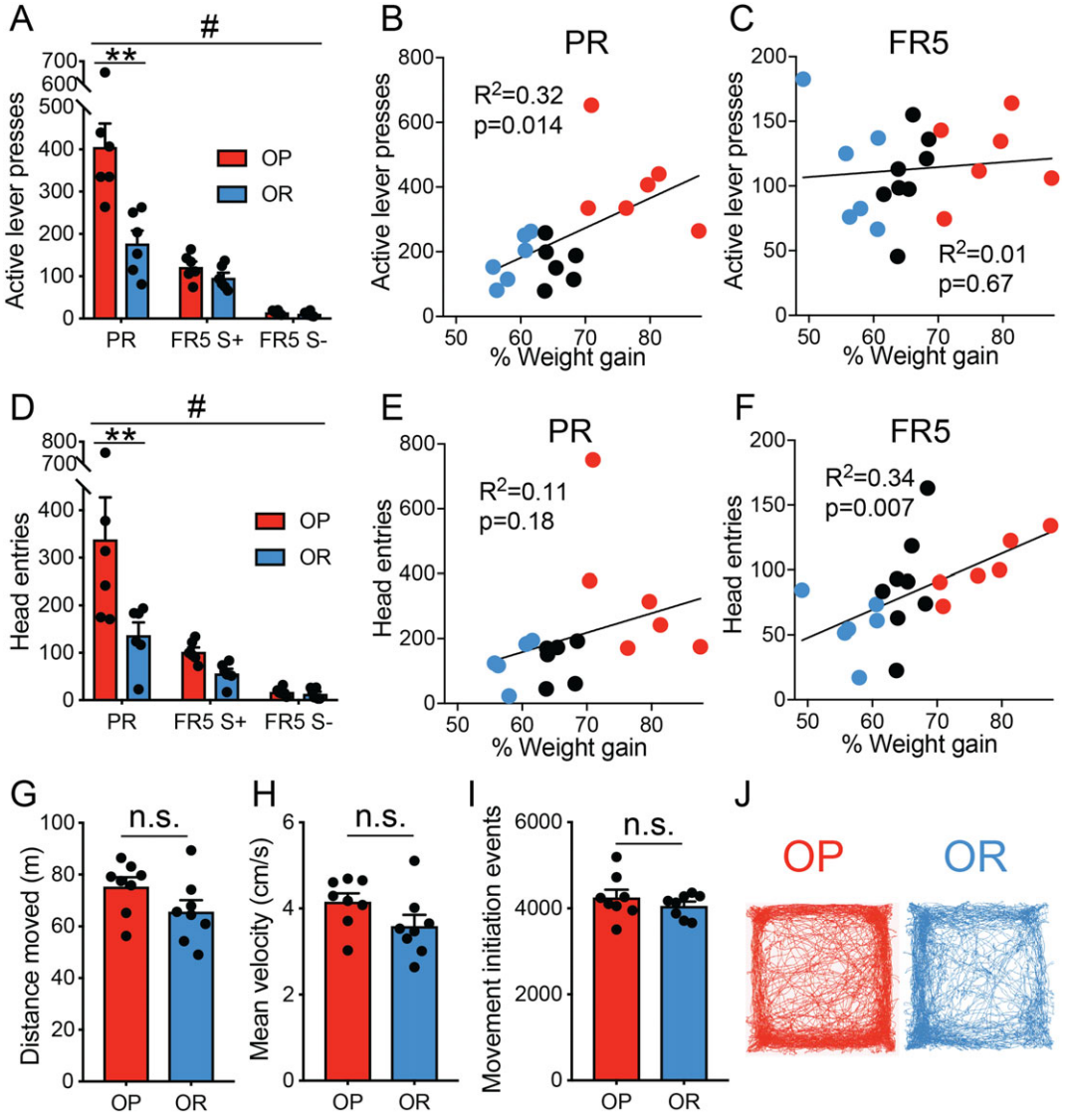

FIGURE 2 Obesity-prone (OP) mice show higher motivation than obesity-resistant (OR) mice toward palatable food after chronic high-fat-high-sugar (HFHS) diet. A, Active lever presses (OP vs OR) in progressive ratio (PR), FR5 S+, and FR5 S− tests after HFHS diet. #$P < 0.001$, two-way analysis of variance (ANOVA), main group effect $F_{(1,\ 30)} = 15.4$. **$P < 0.01$, Sidak's multiple comparison test, $t_{(30)} = 6.02$. Correlations between % weight gain and active lever presses in the PR (B) and FR5 S+ (C) tests. D, Head entries (OP vs OR) in PR, FR5 S+, and FR5 S− tests after HFHS diet. #$P < 0.01$, two-way ANOVA, main group effect $F_{(1,\ 30)} = 7.14$. **$P < 0.01$, Sidak's multiple comparison test, $t_{(30)} = 3.71$. Correlations between % weight gain and head entries in the PR (E) and FR5 S+ (F) tests. There was no difference in the distance moved (G), mean velocity (H), and the number of times mice initiated movement (I) between OP and OR mice after the HFHS diet. J, Representative locomotion plots. N = 6 for all groups

3.1 | Increased motivation for palatable food in obese mice after chronic HFHS diet

Overconsumption of palatable food, in rodents and in humans, may be driven, at least in part, by increased motivation for reward.[10,12] To examine whether OP mice also exhibit increased motivation to obtain palatable food, we compared their performance with that of OR mice. Mice had to press a lever to obtain a pellet (20 mg) of highly palatable food, and the number of lever presses required to obtain a pellet either progressed with performance (progressive ratio [PR]) or was fixed to five lever presses per pellet (FR5). Moreover, in the FR5 test, food was either signaled to be available (FR5 S+) or unavailable (FR5 S−).

Comparing active lever pressing across the three tests reveals a main effect of group—OP mice pressed the lever significantly more compared with OR mice (Figure 2A). The same effect was seen when examining the head entries to the food receptacle (Figure 2D)—OP mice showed a higher rate of head entries. In both parameters, post hoc analyses revealed that the difference between OP and OR mice was significant in the PR test but not in the FR5 S+ or S− test (Figure 2A and 2D). This may imply that the PR test better differentiates between the persistence of OP and OR mice to obtain palatable food.

The persistence to obtain palatable food after the HFHS diet, measured as active lever presses or head entries, generally correlated positively with the level of weight gain (Figure 2B, 2C, 2E, and 2F). This

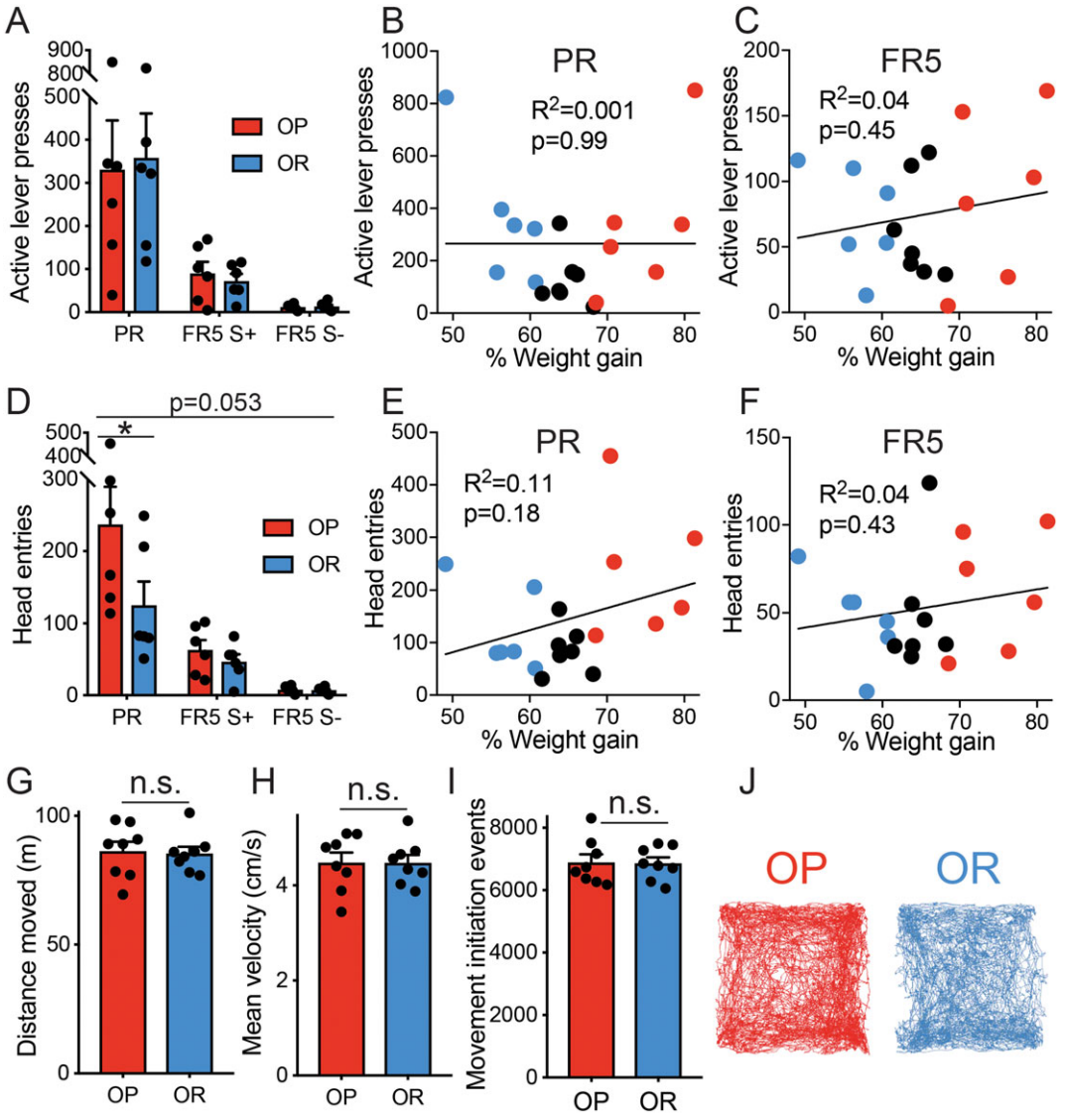

FIGURE 3 Obesity-prone (OP) mice show increased approach to palatable food before exposure to high-fat-high-sugar (HFHS) diet. A, Active lever presses (OP vs obesity-resistant [OR]) in progressive ratio (PR), FR5 S+, and FR5 S− tests before HFHS diet. There was no difference between OP and OR mice in either of the tests. Correlations between % weight gain and active lever presses in the PR (B) and FR5 S+ (C) tests. D, Head entries (OP vs OR) in PR, FR5 S+, and FR5 S− tests before HFHS diet. OP mice showed a higher level of head entries across all behavioral tests ($P = 0.053$, main group effect, $F_{(1, 30)} = 4.035$). Post hoc analysis shows that the difference between OP and OR head entries was significant in the PR test (*$P < 0.05$, Sidak's multiple comparison test, $t_{(30)} = 3.02$). Correlations between % weight gain and head entries in the PR (E) and FR5 S+ (F) tests. There was no difference in the distance moved (G), mean velocity (H), and the number of times mice initiated movement (I) between OP and OR mice before the HFHS diet. J, Representative locomotion plots. N = 6 for all groups

correlation was significantly positive for PR active lever presses (Figure 2B) and FR5 S+ head entries (Figure 2F). There was no correlation between the level of weight gain and lever pressing or head entries in control mice receiving only chow diet (data not shown). Thus, the increased motivation to obtain palatable food seems to depend on prior exposure to that food.

Locomotion tests reveal that OP and OR mice did not differ in their general level of activity (Figure 2G-2J). Thus, the increased actions of OP mice toward food cannot be attributed to a general increase in activity. Likewise, the bigger increase in body weight in OP mice cannot be attributed to a lower level of activity.

3.2 | Is overmotivation for food in OP mice innate or acquired during chronic HFHS diet?

The increased persistence of OP mice may be the cause of their obesity, but it may also be the consequence of the chronic exposure to palatable food. To determine which of the above is a more likely explanation, we trained and tested mice before the beginning of the HFHS diet and compared mice that were later classified as OP mice with those that were eventually classified as OR. Our data show that when it comes to operant behavior (ie, lever pressing for food), OP and OR mice were not different before exposure to the HFHS diet (Figure 3 A; two-way ANOVA, no main group effect) and lever presses did not correlate with final weight gain (Figure 3B and 3C). However, a two-way ANOVA revealed that the more natural food-seeking manifestation, head entries, was higher in OP mice (Figure 3D, $P = 0.053$) even before starting the HFHS diet. Bearing in mind that the effect only trended toward significance, post hoc analyses showed that the difference in head entries was most significant in the PR test (Figure 3D), as also seen after the HFHS diet. Head entries also correlated positively with the final weight gain, although this did not reach significance (Figure 3E and 3F). Finally, open-field tests indicated that the mice did not differ in their locomotion before starting the HFHS diet (Figure 3G-3J). Collectively, these results suggest that while innate food-seeking behavior may already be increased in OP mice before being exposed to the HFHS diet, it fully matures only after chronic exposure to the HFHS diet.

3.3 | Fourteen days of forced abstinence from HFHS food erases the motivational differences between OP and OR mice

Quitting bad eating habits (ie, "going on a diet") is one of the biggest challenges obese individuals face, mainly because of the constant urge to continue eating highly palatable food. One common strategy in treating obesity is to completely prevent obese patients from eating palatable food and help them stick to a balanced diet. Here, we examined whether such strategy would eliminate the differences observed in OP vs OR mice when testing their persistence to obtain palatable food. After the chronic HFHS diet, all mice were switched back to chow for 14 additional days. Then, we examined their motivation to obtain food using the PR test. After 14 days of abstinence, both OP and OR groups lost weight to a similar extent (approximately 7.5%) and thus largely maintained the difference in weight gain (data not shown). In contrast, and unlike our previous results with rats,[10] 2 weeks after switching back to chow, OP mice no longer showed increased persistence in obtaining HFHS food compared with OR mice (Figure 4). This was true both for active lever presses (Figure 4A and 4B) and for head entries (Figure 4C and 4D). Note that although OP mice did not show higher lever presses than OR mice after abstinence, this is not because they restored their baseline lever pressing. On the contrary, lever pressing for both groups was the highest after abstinence (Figure 5A and 5B). Thus, when HFHS food is freely available, OR mice, but not OP mice, maintain lower lever pressing, but when the HFHS food is prevented, both groups increase their lever pressing to a similar level.

3.4 | Head entries are better obesity predictors than lever presses in mice

Comparing OP vs OR mice performance in the PR task across all time points, we found that active lever presses were not significantly different between OP and OR mice (Figure 5A). Rather, as shown in Figures 2–4, they were sensitive to the current diet of the mouse. In contrast, we found that head entries were consistently higher in OP mice across all time points—before, right after, and 2 weeks after the chronic HFHS diet (Figure 5C). Also, head entries showed, similar to lever presses, a trend toward statistically significant main effect of time (two-way ANOVA, $P = 0.053$), with head entries in abstinence being the highest independent of group (Figure 5C and 5D). Thus, head entries may capture better than lever presses the internal urge to consume palatable food in mice.

3.5 | Chronic HFHS diet decreases locomotion and alters movement pattern in both OP and OR mice

So far, we have shown that in each time point, there was no difference in locomotion between OP and OR mice. However, HFHS diet may still have effect on locomotion of both groups. When comparing locomotion of both OP and OR mice before and after the diet, we indeed found that the HFHS diet decreased the distance moved (Figure 6A-6C) and the duration of movement (Figure 6D-6F) in both groups of mice. In both parameters, we found the decrease in locomotion not only at the group level but also when testing the individual mice (Figure 6B, 6C, 6E, and 6F). In addition, the distance moved or the duration of movement (both before and after the HFHS diet) did not correlate with the final weight gain, and the magnitude of the HFHS-induced decrease in each parameter was independent of the final weight gain (Figure S3). In mice fed only on chow, there was a much smaller decrease in the distance moved (Figure 6G) and no change in the duration moving (Figure 6H). Thus, the drastic decrease in locomotion seen in OP and OR mice may be attributed to the chronic exposure to HFHS diet.

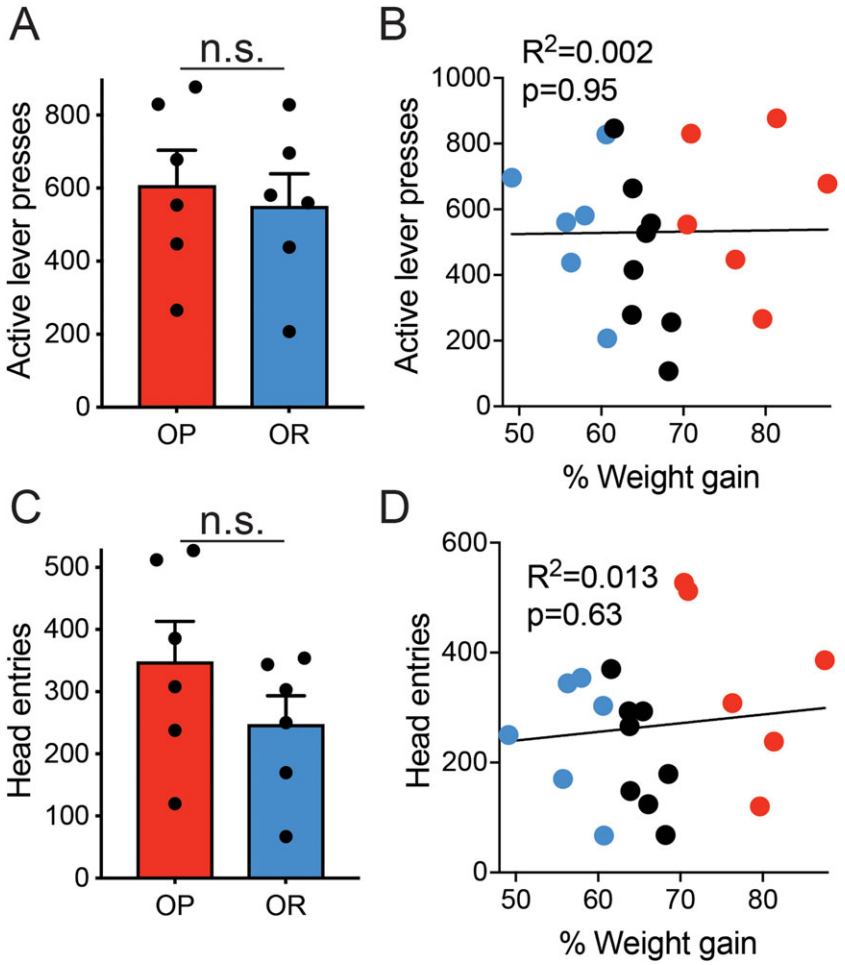

FIGURE 4 Obesity-prone (OP) and obesity-resistant (OR) mice do not differ in their motivation toward palatable food after 2 weeks of abstinence from the high-fat-high-sugar (HFHS) diet. Active lever presses in the progressive ratio (PR) test did not differ between OP and OR mice (A) and did not correlate with % weight gain (B). Head entries in the PR test did not differ between OP and OR mice (C) and did not correlate with % weight gain (D)

Examination of the pattern of movement of mice before and after the chronic exposure to HFHS diet reveals that mice spent significantly less time away from the edges of the test box after the HFHS diet (Figure 6I). There was no difference in this aspect between OP and OR mice. Mice fed only on chow, on the other hand, did not show any difference in movement patterns 3 months after starting the chow diet (not shown). Sticking to the edges of the box may indicate a decrease in exploratory behavior or an increase in anxiety (Crusio,[24] Denenberg,[25] and Kulesskaya and Voikar[26] but see critical review by Ennaceur[27]). Thus, our results show that chronic HFHS diet not only inhibits movement but may also increase anxiety-like behavior, as recently suggested in OP rats.[28]

3.6 | Chronic HFHS diet impairs discrimination between active and inactive levers

Our data show that OP mice are more motivated to seek for palatable food than OR mice, and this was obvious when looking at both head entries and active lever presses. In addition to these measures, inactive lever pressing may also carry information about motivation and cognitive abilities. Inactive lever pressing did not differ between OP and OR mice before they were exposed to the chronic HFHS diet (Figure 7A and 7B). However, when examining inactive lever pressing after the HFHS diet, we found that OP mice pressed also the inactive lever more than OR mice, and this was most significant in the PR task (Figure 7C and 7D). The same was observed even 2 weeks after switching back to chow (Figure 7E and 7F). Across time points, inactive lever pressing seems to increase in OP mice but remains low in OR mice (Figure 7G and 7H).

The increase in both active and inactive lever pressing after exposure to HFHS diet might indicate that HFHS food impairs mice ability to discriminate between the active and inactive levers. To test for that, we measured the ratio between the difference (active − inactive [A − I]) and the sum (active + inactive [A + I]) of active and inactive lever pressing in OP, OR, and control chow mice. First, we found that before the chronic HFHS diet, this lever discrimination ratio was high (Figure 8A, OR = 0.78 ± 0.07, OP = 0.83 ± 0.04, chow = 0.74 ± 0.06) and did not differ between groups. This indicates that all mice had learned the task and there was no dependence between the

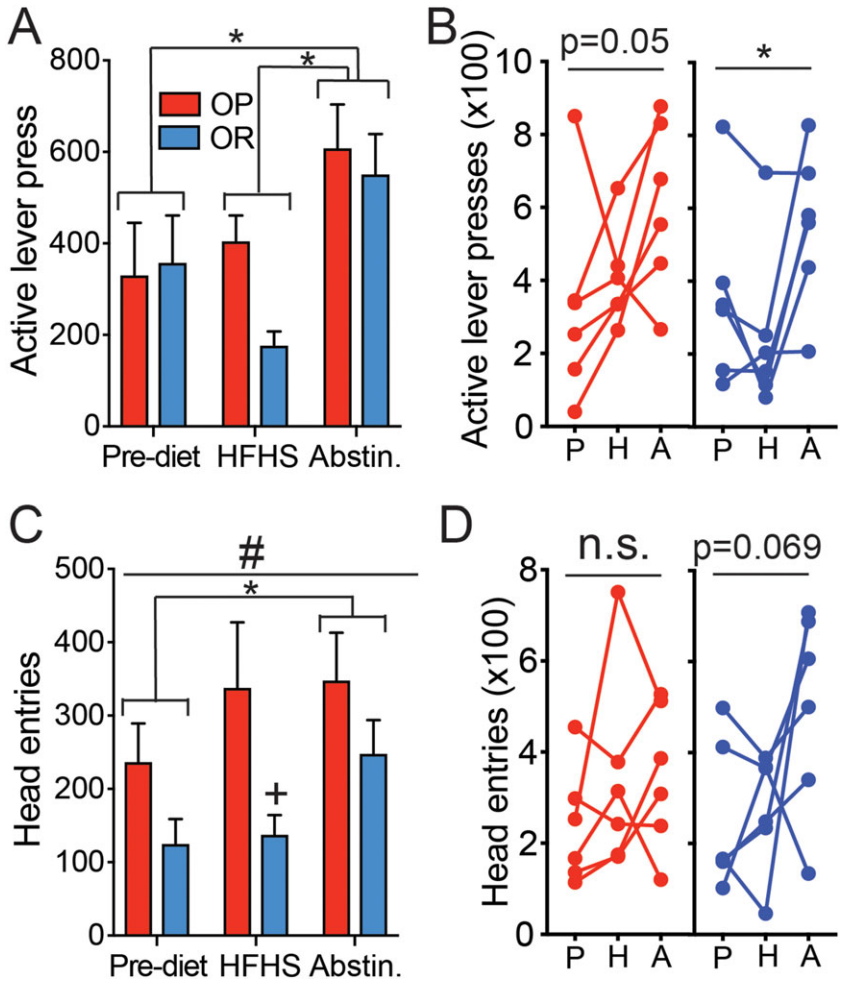

FIGURE 5 Head entries, but not active lever pressing, are consistently higher in obesity-prone (OP) mice compared with obesity-resistant (OR) mice in the progressive ratio task. A, The overall active lever presses in the PR task at three time points of the study did not differ between OP and OR mice (no main group effect with two-way analysis of variance [ANOVA]), but after abstinence, both OP and OR groups increased lever pressing significantly (two-way ANOVA, main time effect, $F_{(2,\ 20)} = 10.2$, $P = 0.0009$), and lever pressing after abstinence was higher than in the two other conditions (Sidak's multiple comparison test, $t_{(20)} = 3.47$, $P < 0.01$ and $t_{(20)} = 4.25$, $P < 0.01$ for abstinence compared with prediet and high-fat-high-sugar [HFHS], respectively). B, Change in active lever presses across the three time points for individual OP (left) and OR (right) mice. In both groups, there was a main effect of time (repeated measures one-way ANOVA, $F_{(2,\ 10)} = 4.1$, $P = 0.05$ for OP mice and $F_{(2,\ 10)} = 8.4$, $P = 0.007$ for OR mice) (P, prediet; H, HFHS; A, abstinence). C, The overall head entries in the PR task across three time points of the study are significantly higher in OP mice compared with OR mice. (#$P = 0.04$, two-way ANOVA, main group effect $F_{(1,\ 10)} = 5.37$; *$P < 0.05$, Sidak's multiple comparison test, $t_{(30)} = 2.55$; +$P < 0.05$, Sidak's multiple comparison test OP vs OR in the HFHS condition). D, Change in head entries across the three time points for individual OP (left) and OR (right) mice. OR mice showed a nonsignificant trend of time (repeated measures one-way ANOVA, $F_{(2,\ 10)} = 3.79$, $P = 0.069$), which was not seen in OP mice ($F_{(2,\ 10)} = 1.34$, $P = 0.3$)

performance on the task and weight gain (Figure 8B). This is also supported by the finding that OP and OR mice needed a similar duration of training on the FR1 task (Figure S4). After 12 weeks of HFHS diet (or chow diet for control mice), the (A − I)/(A + I) ratio dropped for the OP mice and was significantly lower than that of the OR and chow mice (Figure 8C). At this time point, the ratio also negatively correlated with the level of weight gain (Figure 8D). Even 2 weeks after switching back to chow, the ratio was still lower in OP mice (Figure 8E) and negatively correlated with weight gain (Figure 8F). Close inspection of active and inactive lever presses shows that after switching back from HFHS to chow, active lever presses seem to reach a maximum level in both OP and OR groups (Figure 5A, around 600 active lever presses) while inactive lever presses remained low for OR mice but increased gradually for OP mice (Figure 7G and 7H). This indicates that the difference in the discrimination ratio between OP and OR mice stems from the inability of OP mice to discriminate between levers rather than the increased ability of OR mice to perform on the task.

4 | DISCUSSION

Chronic exposure to high caloric palatable food is one of the main causes of obesity. Here, we show that mice with similar initial body weight and genetic background, which were exposed to chronic

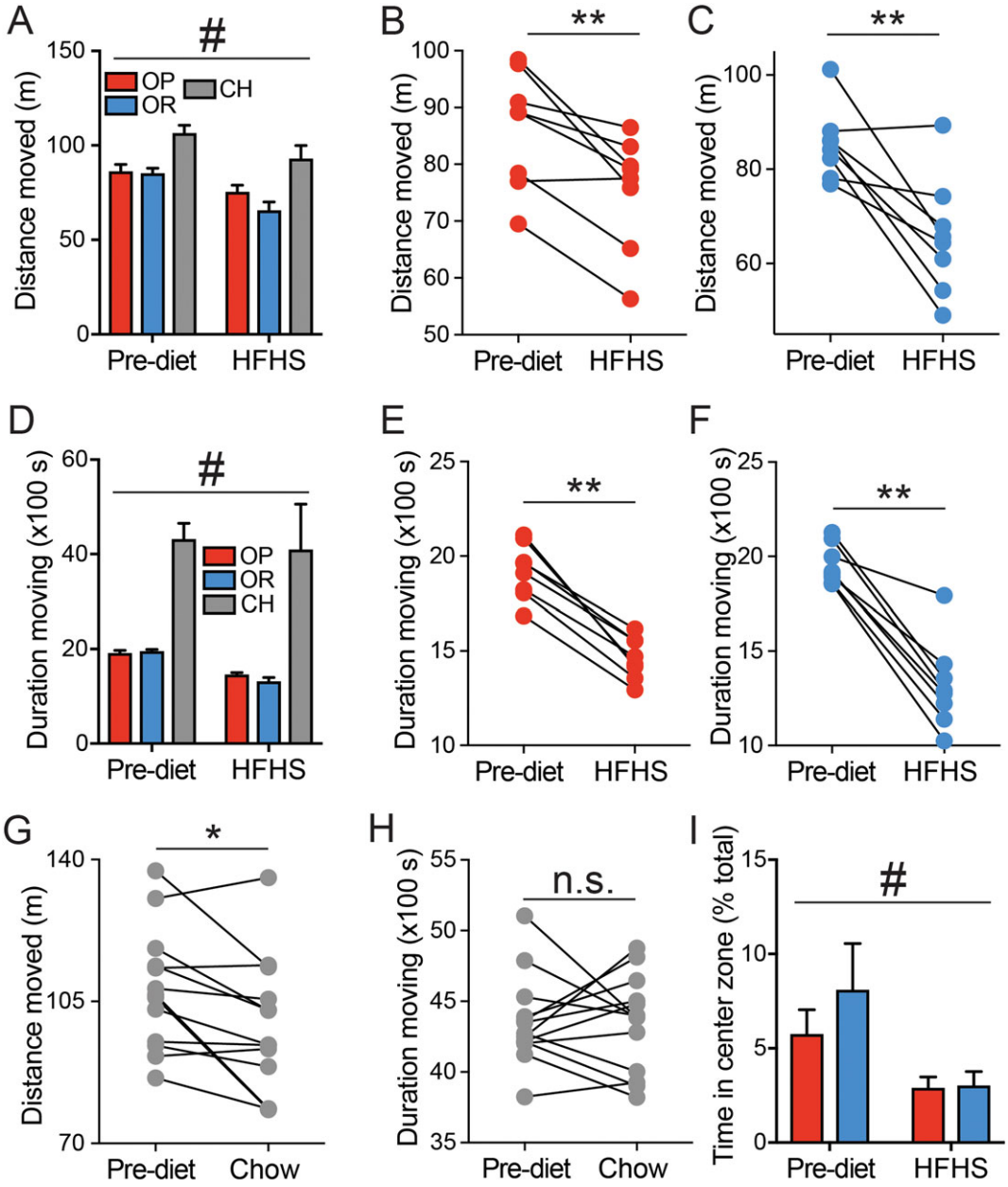

FIGURE 6 High-fat-high-sugar (HFHS) diet reduces locomotion in obesity-prone (OP) and obesity-resistant (OR) mice and changes movement pattern in OP mice. A-C, Chronic HFHS diet reduced the distance moved in both OP and OR mice. D-F, Chronic HFHS diet reduced the duration of time the mice spent in movement both in OP and OR mice. #$P < 0.0001$, two-way analysis of variance (ANOVA) main time effect, $F_{(1, 26)} = 39.26$ and $F_{(1, 26)} = 45.52$ for distance moved and duration moving, respectively). **$P < 0.01$ and *$P < 0.05$, paired two-tailed t tests before vs after the diet. G, Chow-fed mice showed a slight reduction in the distance moved, but to a much lesser extent compared with HFHS-fed mice (−8.60 ± 9.9% and −17.52 ± 12.9%, respectively). H, Chow diet did not affect the duration of moving in a control group (unpaired t test). I, HFHS-fed mice spent less time in the center zone (5 × 5 cm) of the open-field box after the HFHS diet (#$P = 0.03$, two-way ANOVA, main time effect $F_{(1, 10)} = 6.25$)

HFHS diet, gained weight to different extents. We classified these mice into OP and OR groups. By analyzing active lever presses and head entries into food receptacle, we show that exposure to the HFHS diet caused significant differences in motivated food-seeking behavior, where OP mice showed higher persistence toward food than OR mice. We also show that the HFHS diet impaired locomotor activity in all mice, but affected cognitive aspects of food-seeking behavior selectively in OP mice.

4.1 | Is increased food seeking in OP mice a cause or consequence of obesity?

Our main finding is that after chronic exposure to HFHS food, OP mice are significantly more motivated to obtain palatable food compared with OR mice (Figure 2). When examining the behavior of the same mice before the exposure to HFHS, we found that while operant food seeking was similar between all mice, the more natural

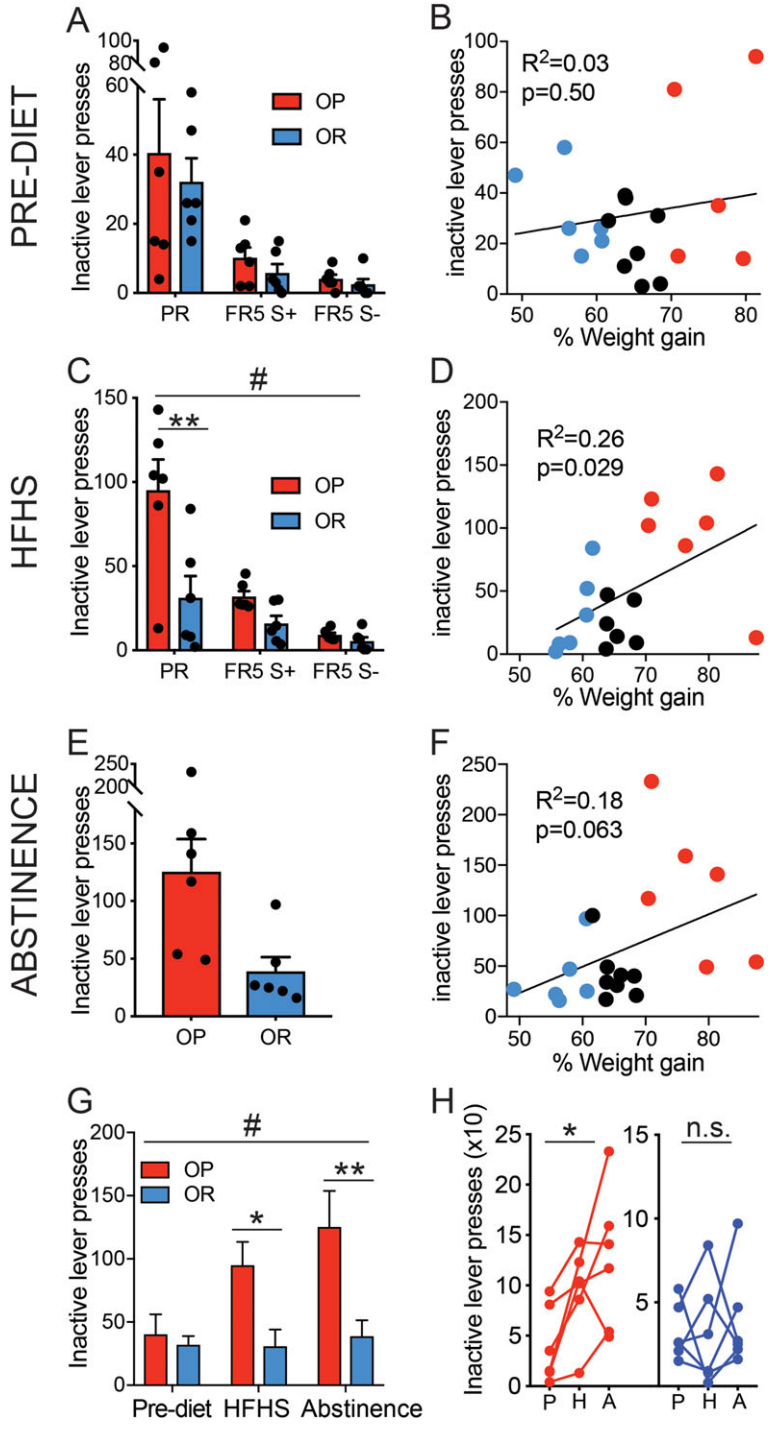

FIGURE 7 Obesity-prone (OP) mice overpress the inactive lever after chronic exposure to and abstinence from high-fat-high-sugar (HFHS) food. A, B, Prediet behavior. A, Inactive lever presses (OP vs obesity-resistant [OR]) in PR, FR5 S+, and FR5 S− tests before HFHS diet. There was no difference between OP and OR mice in either of the tests. B, Correlations between % weight gain and inactive lever presses in the PR test. C, D, Behavior after chronic HFHS diet. C, Inactive lever presses (OP vs OR) in PR, FR5 S+, and FR5 S− tests after HFHS diet. #$P < 0.001$, two-way analysis of variance (ANOVA), main group effect $F_{(1,\ 30)} = 13$. **$P < 0.01$, Sidak's multiple comparison test OP vs OR, $t_{(30)} = 4$. D, Correlations between % weight gain and inactive lever presses in the PR test. E, F, Behavior after abstinence from HFHS food. E, Inactive lever presses in the PR test were higher in OP mice compared with OR mice. *$P < 0.05$, unpaired two-tailed t test, $t_{(10)} = 2.8$. F, The correlation between inactive lever presses and % weight gain was positive and almost reached significance ($P = 0.063$). G, Across all time points, OP mice gradually increase the level of inactive lever pressing while OR mice maintain a steady level. #$P < 0.05$, two-way ANOVA, main group effect, $F_{(1,\ 10)} = 9.19$. *$P < 0.05$, Sidak's multiple comparison test OP vs OR, $t_{(30)} = 2.67$. **$P < 0.01$, Sidak's multiple comparison test OP vs OR, $t_{(30)} = 3.59$). H, Change in inactive lever presses across the three time points for individual OP (left) and OR (right) mice. OP mice showed a significant main effect of time (repeated measures one-way ANOVA, $F_{(2,\ 10)} = 6.55$, $P = 0.02$), which was not seen in OR mice ($F_{(2,\ 10)} = 0.14$, $P = 0.9$) (P, prediet; H, HFHS; A, abstinence). N = 6 in all groups

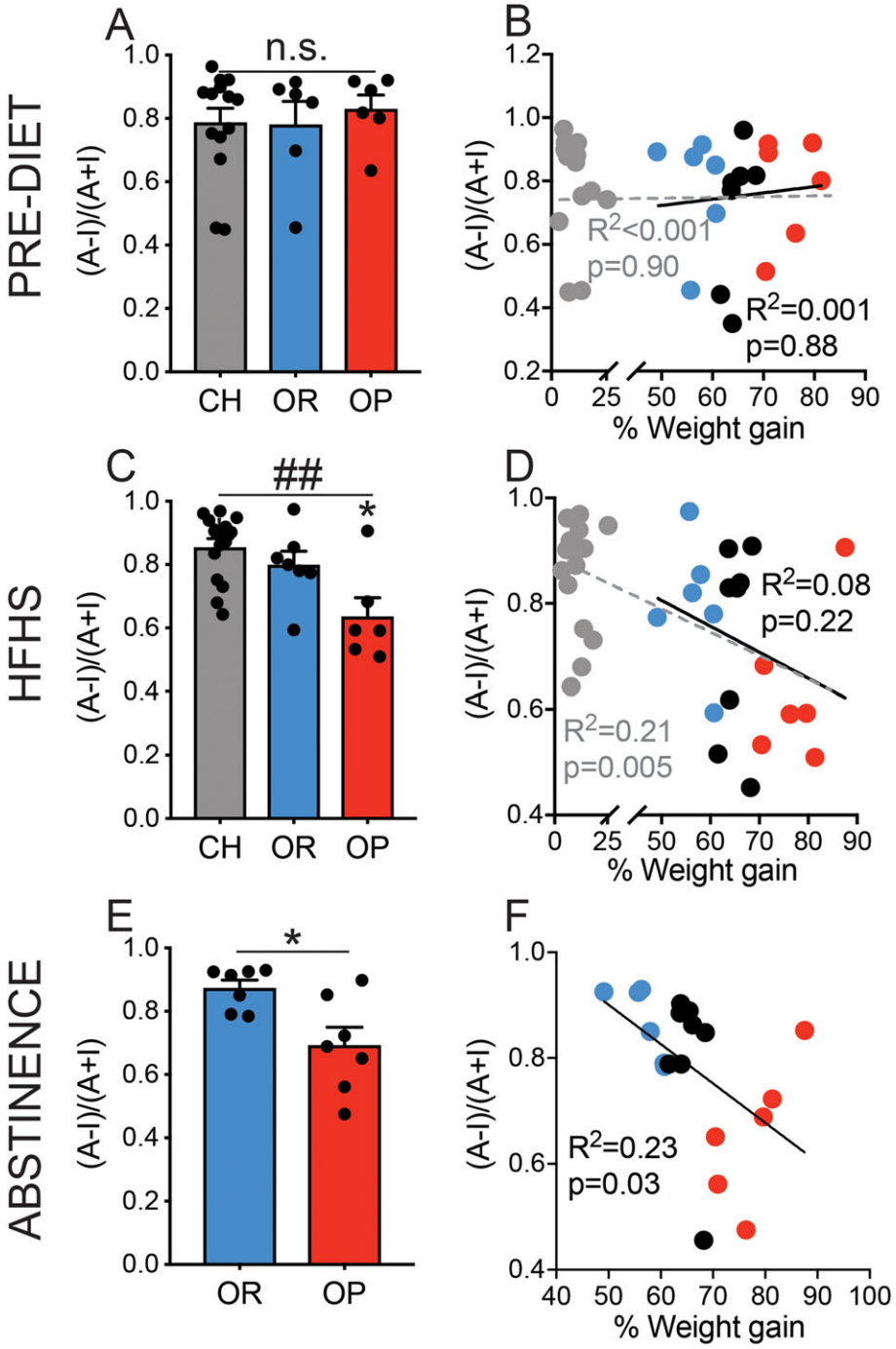

FIGURE 8 Obesity-prone (OP) mice show persistent decrease in the discrimination between active and inactive levers after chronic high-fat-high-sugar (HFHS) diet. The ratio between the difference (active − inactive [A − I]) and the sum (active + inactive [A + I]) of active and inactive lever pressing was used as a measure of discrimination between the levers in a progressive ratio task. The (A − I)/(A + I) ratio was not different between chow (CH, ratio = 0.74 ± 0.06), OP (ratio = 0.83 ± 0.04), and obesity-resistant (OR, ratio = 0.78 ± 0.07) mice (A) and did not correlate with weight gain (B) before the exposure to the HFHS diet. Gray and black numbers in panels B and D refer to the correlation with and without the chow group, respectively. C, After chronic HFHS diet, the discrimination ratio decreased in OP mice (ratio = 0.64 ± 0.06) and differed significantly from both OR (ratio = 0.80 ± 0.04) and chow (0.86 ± 0.03) mice. ##P < 0.01, one-way analysis of variance (ANOVA) main group effect, $F_{(2, 25)}$ = 7.63. *P < 0.05 compared with OP mice, Tukey's multiple comparison test. D, The ratio was inversely correlated with weight gain, and this correlation was significantly different from zero (with chow mice included). E, After 2 weeks of abstinence from HFHS food, the discrimination ratio remained significantly lower in OP mice (0.69 ± 0.06) compared with OR mice (0.87 ± 0.03) and was inversely correlated with weight gain (F). *P < 0.05, OP vs OR, unpaired two-tailed t test. N = 15 in the chow group; N = 6 in OP and OR groups in (A), (C), (E); N = 20 without chow; and N = 35 with chow in (B), (D), (F)

manifestation of food seeking, namely head entries to the food receptacle, was already higher in OP mice (Figure 3). This suggests that on average, mice that will eventually gain the most weight if exposed to chronic HFHS diet have a high motivation to consume palatable food that is independent of experience. This increased motivation does not affect body weight if rewarding food is not available—the development of body weight and the daily caloric consumption were similar between groups before the HFHS diet (Figure 1). Thus, we conclude that a prediet high motivation to consume palatable food may be essential but not sufficient for the development of obesity. Only in a setting where HFHS is freely available (as is the case in the modern world) will this high motivation for palatable food lead OP individuals to develop obesity.

4.2 | OR, but not OP mice, succeed in controlling their motivated food-seeking behavior

Following exposure to the HFHS diet, OP mice demonstrated a significantly higher motivated food-seeking behavior compared with OR mice (Figure 2). Intuitively, one would think that OP mice increase more strongly than OR mice their lever presses for palatable food after the chronic exposure to HFHS diet. However, a closer look at the results reveals that the main source for the difference between the two groups is more likely to be a decrease in food seeking of the OR mice following the HFHS diet (−50%) rather than an increase in OP mice (+23%). This means that OP and OR mice will both show high motivation if palatable food is scarce, but when HFHS is available, chronically OP mice will maintain high consumption while OR mice will decrease food consumption. Thus, OR mice, in contrast to OP mice, have a seemingly intact ability to control their food-seeking behavior when facing chronic exposure to HFHS food. This, in conjunction with the increased basal motivation to seek for rewarding food in OP mice discussed above, may underlie the high caloric consumption of OP mice in the home cage.

4.3 | Head entries vs active lever presses as a measure of motivated eating

Head entries into the food receptacle (HEs) and active lever presses (ALPs) were both used as a measure of motivated food-seeking behavior; however, the trends in these two behaviors were not exactly similar. Most notably, HEs were higher in OP mice across all time points examined while ALPs became higher in OP mice only after chronic exposure to HFHS diet (Figure 5). This is particularly important when examining the behavior of mice before the exposure to the HFHS diet. One possible reason for this discrepancy between HEs and ALPs is that HEs capture consumption-related motivation and are presumably a more natural behavior for mice than the instrumental ALPs,[29,30] particularly in appetitive behavior.[31] Thus, HEs may reflect in a more direct manner the inner motivation of the mice to obtain palatable food. In addition, the food receptacle is likely to smell of palatable food and so may attract all mice, but more efficiently mice who are more eager to consume palatable food. Regardless of the mechanism, the mere fact that HEs are higher in OP mice already before exposure to HFHS suggests that the HE measure might serve (in mice) as a predictive indicator on whether a mouse would be prone to develop obesity or rather resist it, if exposed to HFHS diet.

4.4 | Locomotion vs motivated overeating in determining body weight gain

One might claim that the higher degree of lever pressing in OP mice compared with OR mice following the chronic HFHS diet could have resulted from a higher level of general locomotion of OP mice. Our results, however, corroborate a recent study[11] and show a *decrease* in the degree of general locomotion of both OP and OR mice following calorie-rich diet (Figure 6). Furthermore, there was no significant difference between the two groups after the HFHS diet. In their study, Friend et al showed that mice fed chronically with high-fat diet, compared with chow-fed mice, show reduced striatal D2 receptor availability, which in turn leads to reduced general locomotion. However, dopamine signaling is involved not only in motor control but also in reward-driven behavior. In our study, having differentiated OP from OR groups among the HFHS-fed mice, we found that while OP mice exhibited higher persistence to obtain food in comparison with OR mice, there was no difference in the locomotion between the two groups. This suggests that while the difference in body weight gain *between* chow-fed mice and mice fed with calorie-dense food may be strongly affected by locomotor activity, the differences in body weight gain *within* the group fed with calorie-dense food depend more on the motivation to seek for the food and not on energy spending. Therefore, we conclude that in a situation where the entire population is chronically exposed to calorie-dense food, the differences in body weight gain between individuals depend more on the motivation to obtain the food rather than locomotor activity.

4.5 | Cognitive implications of HFHS diet on mice

Interestingly, following the HFHS diet, not only did OP mice show higher persistence in obtaining food, but they also demonstrated impaired ability to discriminate between the active and inactive levers. During training, and before the exposure to the HFHS diet, all the mice learned quickly to distinguish between the active and inactive levers. This was noticed by the high ratio between the difference (A − I) and the overall (A + I) lever presses (Figure 8A). Nevertheless, after exposure to the chronic HFHS diet, a significant difference in this ratio emerged between the OP and OR mice—OP mice exhibited relatively impaired discrimination between the two levers in comparison with OR mice. Importantly, the HFHS diet did not impair the performance on the task in OR mice, as their ratio remained high and similar to that of chow-fed mice (Figure 8C).

The decrease in the discrimination ratio may point to a cognitive impairment in OP mice that is induced only in the most extreme cases of HFHS food consumption. The effect of a calorie-dense diet on

cognitive performance is not clear yet. Previous studies point out that a calorie-dense diet may induce cognitive impairments in juvenile mice,[32,33] rats,[34] and humans.[35] These impairments include deficits in learning, working memory, emotional memory, and more.[32-38] Our work shows that if indeed obese individuals suffer from cognitive deficits, these deficits are likely to be caused by the diet and do not exist prior to the diet. Unfortunately, we also show that the cognitive deficits persist even after prolonged abstinence from the calorie-dense diet. More work is needed to understand the roots of these HFHS diet-induced cognitive deficits and attempt to restore normal cognitive functioning.

4.6 | Mice vs rats

Previous studies on the link between food motivation and the development of obesity have used mostly rats as the animal model[10,12,39-41]. These studies have found increased motivation for palatable food in rats that developed obesity[10,12,39] or that were selectively bred as OP.[41] This high motivation for palatable food was persistent even after restoring normal diet[10] and was found to some extent even before developing obesity.[40,41] Here, we used inbred C57bl6/J mice, which showed some differences and some similarities to the observations in rats. First, although OP mice, like OP rats, did show higher motivation during the HFHS diet, this disappeared after 2 weeks of abstinence (Figure 4). One possible explanation for this is that mice and rats react differently during abstinence from palatable food. Indeed, while OP rats maintain a steady body weight during abstinence,[10] OP and OR mice showed a significant loss of weight (approximately 7.5%). Another difference between mice and rats linked to their size is the amount of weight they gain during the HFHS diet. While rats fed on chow gain weight to the same extent as OR rats,[10,41] mice fed on chow showed a significantly lower weight gain (Figure 1) compared with OR mice. Collectively, it seems that HFHS diet affects more profoundly the body weight of mice, both when the diet begins and when it is ended. Lastly, the behavior of rats is more robust—OP rats show higher motivation for palatable food in almost every test used.[10,40,41] In contrast, the difference between OP and OR mice was significant in the PR test but showed only trends in the FR5 tests. Thus, when using mice to study OP vs OR groups, the behavioral test should be considered carefully.

4.7 | Statistical analysis

Statistics were performed using GraphPad Prism 7.04 (GraphPad Software Inc., San Diego, CA). Specific statistical tests are indicated for each experiment in the figure legends.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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