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Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area

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Abstract

Voluntary wheel running (WR) is a form of physical activity in rodents that influences ingestive behavior. This study examined the effects of WR on dietary preference and the potential role of leptin in mediating these effects. In a two-diet choice paradigm in which both palatable high-fat (HF) food and standard laboratory chow were provided *ad libitum*, rats displayed a strong preference for the former and chose to eat almost exclusively the HF diet over chow in sedentary conditions. With free access to running wheels, however, rats exhibited no preference for the HF food and consumed equal gram amounts of both chow and HF diets. The total daily caloric consumption during WR in the dietary choice protocol was equivalent to the amount of calories consumed daily by WR rats with only chow or only HF diet available, yet significantly less than sedentary chow caloric consumption. Two days after initiating WR, leptin signal transduction was examined in multiple selected brain sites following leptin injection into the third cerebral ventricle. The maximal leptin-stimulated STAT3 phosphorylation was enhanced only in the ventral tegmental area (VTA), but not in the arcuate nucleus, lateral hypothalamus, dorsal medial or ventral medial hypothalamus, or substantia nigra. In conclusion, wheel running appears to act either as an independent reinforcing factor or as a more favored activity to substitute for the consumption of a palatable HF diet, thus eliminating the preference for the HF food. Moreover, WR enhances leptin signaling specifically in the VTA, suggestive of a WR-evoked mechanism of heightened leptin function in the VTA to curb the drive to consume palatable HF foods.

Keywords

leptin; dietary selection; high-fat feeding; voluntary wheel running

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1. Introduction

Ingestive behavior is governed by complex interdependent factors including both caloric need and the rewarding properties of food [1]. High-fat (HF) foods have strong sensory appeals with reinforcing values, and hence are naturally rewarding. When allowed to choose, rodents consume very little of a nutritiously adequate, low-fat chow when HF foods are also available [2]. In humans, individual differences exist in the perception of reinforcing values of food with obese individuals displaying a stronger preference for diets high in fat and carbohydrates relative to non-obese individuals [3].

Voluntary wheel running (WR) constitutes an activity behavior in rodents that also affects ingestive behavior. Accumulating evidence demonstrates shared neuro-behavioral characteristics of wheel running rodents with either drug or foods as reinforcers, suggesting WR activity itself to be both naturally rewarding and reinforcing [4,5]. In one case for example, the reinforcement value of food was reduced in rats with prior WR while the reinforcing worth of running increased with food deprivation [4]. Little is known in regards to any relationship between voluntary WR and dietary preference, but this subject has been examined with other types of exercises. For instance, female Sprague Dawley rats placed on a macronutrient self-selection diet for 15 weeks while undergoing treadmill exercise training decreased dietary fat selection without changing daily energy intake or consumption of carbohydrates and protein [6]. Exercise is also reported to increase the proportion of fat utilization during short-term consumption of a HF diet [7].

Leptin is an essential hormone in the regulation of both ingestive behavior and energy expenditure, thus playing a critical role in energy homeostasis [8]. Recently, leptin function has been linked to the mesolimbic dopamine system in the ventral tegmental area (VTA) of the midbrain reward circuitry [9,10] and to a regulatory role in propensity for WR in rats [11]. Presentation of highly palatable foods induces robust dopamine release from the mesolimbic reward circuitry, and this release enhances an animal's motivation to obtain food rewards [12]. Leptin is reported to directly attenuate activity of dopamine neurons in the VTA, and leptin receptor knockdown in the VTA increases sensitivity to sucrose consumption [10]. In addition, leptin tempers other addictive behaviors such as heroin seeking [13] that also involves the mesolimbic dopamine pathway. Besides its effects on ingestive behavior and drug addiction, we recently demonstrated that leptin overexpression in the CNS increases WR activity in rats and a partial blockade of leptin receptors decreases spontaneous WR [11].

Several studies indicate the mesolimbic dopamine-opioid system mediates aspects of WR behavior [5,14–16]. Conceivably, WR modulation of ingestive behavior may converge with leptin counter-regulatory action on ingestive behavior at the VTA via the dopaminergic pathway. This study is designed to test the possibility that WR substitutes as a preferred activity over diet selection behavior and hence curbs the preference for HF food in a diet-selection paradigm. Moreover, because increasing evidence indicates that WR enhances brain leptin signaling [17,18], this study thus, also seeks to investigate if WR effects on dietary selection are associated with increased leptin signaling specifically in the VTA. To these ends, we examined the influence of WR on dietary selection between a normal chow and a HF diet in rats and any associated changes in leptin signaling in variety of brain regions including the VTA, substantia nigra (SN), arcuate nucleus (ARC), as well other regions of the basal medial hypothalamus.

2. Material and methods

2.1 Experimental animals

Six-month-old male F344 × Brown Norway (F344×BN) rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Upon arrival, rats were examined and remained in quarantine for one week. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals and protocols approved by the University of Florida IACUC committee. Rats were housed individually with a 12:12 h light:dark cycle (07:00 to 19:00 hr). During the experimental period, rats were fed either a standard rodent chow (17% kcal from fat, 58% kcal from carbohydrate, 25% kcal from protein, 3.1kcal/g, diet 7012, Harlan Teklad; Madison, WI) or a HF diet (60% kcal from fat, 7% sucrose, 20% kcal from carbohydrate (including 7% of the total kcal as sucrose), 20% of kcal from protein, 5.24 kcal/g, D12492, Research Diets, New Brunswick, NJ) or provided with both diets *ad libitum* in the dietary choice protocol.

2.2 Experimental design

This study consisted of four experiments. The first experiment comprised rats (n=6) accustomed to normal chow food and then provided both chow and HF food for one week. At this point, access to running wheels was given to all the rats for an additional week while continuing the availability to both diets. In the final 2-week phase of the experiment, the running wheels were removed but choice between the two diets remained.

In the second experiment, rats (n=10) were accustomed to chow then provided running wheels for 3 days while continuing on the chow diet.

In the third experiment, the rats (n=6) were accustomed to the HF diet for two weeks until the point when consumption of the HF food was stabilized. At this time, running wheels were introduced for one week. During the second week, chow diet was introduced in addition to the HF diet and free access to running wheels. In the last week of this experiment, the running wheels were removed, but the two diets remained for continued monitoring of the dietary choice.

The fourth experiment was similar to the first in that animals were accustomed to normal chow food first and then provided both chow and HF food. However, only half the animals were introduced to running wheels and the other half remained sedentary. Two days later, the rats in both the sedentary and WR groups were administered either artificial cerebral spinal fluid (vehicle) or leptin (1ug) by injection into the third cerebral ventricle and killed one hour later for assessment of leptin signaling in selected brain regions (n=6/group). Body weight, food intake, and the extent of wheel running (WR) were recorded daily throughout the experiments.

2.3 Dietary selection

Rats were provided access to standard chow, a HF diet, or both simultaneously. When provided both, food consumption of both diets was determined separately by weight of food consumed. The position of the food trays containing the chow and HF food was alternated daily. Spillage of food was accounted for in calculating food consumption.

2.4 Leptin administration

A single dose of leptin (1 µg) was injected into the third cerebral ventricle as previously described [19]. The coordinates for injection are 1.3mm anterior to Bregma, 9.4mm ventral from the skull surface, at an angle of 20 degrees anterior to posterior. Rats were killed one hour later.

2.5 Wheel running

Rats were housed in cages equipped with Nalgene Activity Wheels (1.081 meters circumference, Fisher Scientific, Pittsburgh, PA) and allowed free access to the wheel. Each wheel was equipped with a magnetic switch and counter. The number of revolutions was recorded daily.

2.6 Tissue harvesting

Rats were killed by thoracotomy under 150-mg/kg pentobarbital anesthetic. The circulatory system was perfused with 20ml of cold saline, and the brain removed for sectioning. Brain sections were cut using a micrometer controlled tissue slicer (Stoelting Co, Wood Dale, IL). Each coronal brain slice was cut in reference to the location of Bregma to a width of 2 mm. The section from -5.0 to -7.0 mm contained the VTA and the section from -1.5 to -3.5 mm contained the Arc, VMH, DMH and LH. The sections were placed into a petri dish containing cold saline. With the aid of a dissecting microscope, bilateral micro-punches of 1mm in diameter (Stoelting Co, Wood Dale, IL) were removed. Each set of micro-punches was immediately placed into ice-cold homogenization buffer (10mM Tris-HCl, 2% SDS). Following brief sonication, an aliquot of the homogenate was saved for determination of protein concentrations by the DC protein assay kit (Bio-Rad, Hercules, CA) while the rest of the homogenate was stored at -80°C until use.

2.7 Western analysis

Protein homogenate (20 μg) was separated on a SDS-PAGE gel and electro-transferred to nitrocellulose membranes [19]. Immunoreactivity was assessed with antibodies specific to phospho-tyrosine 705 of STAT3, either phosphorylated or unphosphorylated STAT3 (Cell Signaling, Danvers, MA),

2.8 Statistical analysis

Data were analyzed by one-way ANOVA. When the main effect was significant ($p < 0.05$), Tukey post-hoc test was applied to determine individual differences between means. A p -value of less than 0.05 was considered significant.

3. Results

The influence of WR on dietary selection was examined in rats either accustomed to chow food or HF food.

3.1 Rats accustomed to chow food

Rats consumed a steady amount of chow food of approximately 20.4 ± 0.5 g/day. When the HF diet (60% kcal from fat, 7% sucrose, 20% total carbohydrate, 5.24 kcal/g) was introduced besides the constantly available chow food, there was an immediate hyperphagia as measured by either grams (Fig. 1A) or kcal consumed (Fig. 1B). Moreover, the rats consumed almost exclusively the HF food during this phase of the experiment (Fig. 1A). On the evening of day 7, rats were allowed free access to running wheels. Day 8 marked the first day that food consumption was determined in the presence of running wheels. On this day, the rats continued to eat HF food, but they also began to eat chow food. By day 9, however, they no longer demonstrated a preference for the HF food, consuming equal gram amounts of both chow and HF diets (Fig 1A). This pattern of consumption continued until the running wheels were removed from the cages, and afterwards, food intake reverted back to the pre-WR pattern of consumption of only the HF food (Fig 1A).

The caloric density of the chow and HF diets differ (3.1kcal/g for chow and 5.24kcal/g for HF). When total caloric consumption was examined, introduction of the HF diet induced an initial hyperphagia that partially normalized by day 4 (Fig. 1B), but remained 30% greater than caloric intake of chow consumption (Table 1). There was a second acute and minor hyperphagia following the introduction of the running wheels due to an increase in chow consumption without a commensurate decrease in HF food. This occurred only on the first day of WR. By the second day, there was a rapid drop in HF food intake and a corresponding decrease in total caloric consumption during the remaining one-week WR period at a level significantly less than the sedentary chow caloric consumption (Table 1). After removal of the wheels, caloric consumption returned to the HF (plus chow) intake level observed from day 4 to 6 during the initial diet selection phase prior to introduction of running wheels (Fig. 1B, and Table 1).

In a separate group of age- and weight-matched rats accustomed to chow, we examined the caloric intake following introduction of running wheels. There was an immediate 10% decrease in consumption on the first day of wheel running ($P = 0.044$) and an additional reduction at day 2 that was stabilized through day 3 (Table 1, Experiment 2).

Body weight during the different phases of experiment 1 followed the pattern of total caloric consumption with a tendency for higher growth rates during the high consumption period (HF/ chow choice and no WR) compared with the periods with low consumption (chow only or HF/ chow choice plus WR, Fig. 1C).

3.2 Rats accustomed to HF food

One explanation for the apparent loss of the dietary preference could be a simple consequence of the WR-induced decrease in consumption of HF food. To further examine this issue, we first accustomed the rats to the HF food in the absence of the chow diet. The conditioning continued beyond the initial HF-induced hyperphagic phase to a point when consumption of HF food was relatively steady (Fig. 2A, days -4 to 0), but considerably greater than with just chow consumption ($P < 0.0001$, Table 1, chow sedentary vs HF sedentary). Running wheels were provided beginning at day 0 (Fig. 2A), and similar to that observed in the first experiment, WR decreased HF food intake by greater 50% (Fig. 2, Table 1). However, in contrast to the first experiment in which both HF and chow foods were available, only the HF diet was present during this phase (Fig. 2A, days 0 to 7). In the next phase of the experiment, as WR continued, chow was introduced besides the available HF food. There was an immediate decrease in HF consumption as well as an increase in chow consumption (Fig. 2A, days 8–14). However, total caloric consumption was unchanged, indicating that the reduction in caloric consumption derived from HF intake was compensated for by an equal increase in caloric consumption of the chow diet (Fig. 2B, Table 1). On a gram basis, the rats ate more chow than HF food (Fig. 2A, days 8–14). On a caloric basis, the rat consumed similar amounts of chow and HF (197 ± 25 vs. 173 ± 27 kcal/week, respectively). When the running wheels were removed commencing at day 14, dietary selection reverted back to the pre-WR pattern with the rats eating only the HF diet by the end of the experiment (Fig. 2A), and the total caloric consumption was similar to that prior to day 0 (Fig 2B, Table 1). Body weight, as in experiment 1, mostly paralleled changes in total caloric consumption (data not shown).

3.3 Wheel Running

The extent of WR during the dietary choice phase of Experiment 1 (for reference see Fig. 1) and during the HF only phase of Experiment 3 (for reference see Fig. 2) was not different (317 ± 21 and 329 m/day ± 26 , respectively). This level of WR is typical for this age and strain of rat [20] and is considerable less than observed in Sprague Dawley rats [18]. The amount of HF food consumed during the WR period in the diet-selection phase was inversely correlated with the extent of WR, but amount of chow consumed was not (Fig. 3).

3.4 Leptin signaling in the VTA

To evaluate whether WR is associated with increased leptin signaling in selected brain regions, we repeated the experiment described in Fig. 1, where rats were first accustomed to chow, then permitted a choice between HF and chow food (Fig. 4 top, days -2 to 0), and then followed by the introduction of running wheels (Fig. 4, top, day 0). Daily WR was similar to that in the first two experiments (275 ± 32 m/day). In this experiment, two days after introducing running wheels, the rats were administered leptin (1 μ g) or vehicle by injection into the third cerebral ventricle and killed 1 hour later. Leptin signaling was examined in six different brain regions, arcuate (ARC) nucleus, ventral tegmental area (VTA), lateral hypothalamus (LH), dorsal medial hypothalamus (DMH), ventral medial hypothalamus (VMH) and substantia nigra (SN). Leptin increased STAT3 phosphorylation by 3 to 4 fold over vehicle in the sedentary rats in all six brain regions tested (Fig. 4, bottom and Fig. 5). However, wheel running further elevated this signaling by greater than two-fold only in the VTA (Fig. 4, bottom), but not in any other tissues (Fig. 5) including the ARC (Fig. 4, bottom). Body weight at death was not significantly different between the various groups (490 ± 25 , sedentary control; 484 ± 15 , WR control; 501 ± 12 , sedentary leptin; 493 ± 17 , WR leptin).

4. Discussion

This report examined the extent by which voluntary WR influenced ingestive behaviors with respect to selection between HF food and chow, and revealed two salient findings. First, WR obliterated the preference for HF food in an HF/Chow choice paradigm. When facing such a choice in the absence of running wheels, rodents scarcely consumed the nutritiously adequate, low-fat chow food. On the contrary, rats with free access to running wheels consumed equal gram amounts of both chow and HF foods. Second, wheel running and the WR-mediated elimination of the preference for HF food were associated with enhanced leptin signaling specifically in the VTA region of the brain.

These findings are consistent with and in support of the idea that wheel running, in-and-of itself, substitutes for behaviors leading to the dominant selection of a HF diet. When chow-fed rats are first introduced to HF food, they display a hyperphagia with respect to total caloric consumption that persists for a variable period of time. As mentioned earlier, these rats choose to consume almost exclusively the HF food when presented with both the chow and HF diets. WR evidently dampens the desire to consume the more palatable HF food: rats with access to running wheels and to both chow and HF foods consumed equal gram amounts of both types of food. WR not only eliminated food preference between chow and HF, but also diminished the caloric consumption of chow alone, HF alone or both foods present such that the total daily caloric consumption associated with running wheels was equivalent regardless of foods presented. Thus, there appears to be two apparent consequences of this short-term WR; the first is an overall decrease in caloric consumption. Since WR increases physical activity hence energy expenditure, it is unclear why the WR rats did not elevate their food intake. Some mice tend to adjust to the new energy demand by eating more when wheel running [21,22], whereas some rats undergoing long-term exercise appear to maintain the pre-running food consumption level [18,23,24]. F344 $\times$ BN rats run considerably less than age-matched Sprague Dawley rats [18], thus their respective activity involves different energetics. The increase in energy expenditure accompanying a high degree of running may induce a higher compensatory demand for greater food consumption. Short-term and mild levels of WR, however, may be insufficient to generate a demand for increased food intake, and the effects of WR on behaviors such as diet selection are more easily detected under this circumstance. Although, we cannot rule out the potential impact of variations in nutrient composition or energy density of the two diet on the observed behaviors, the WR-mediated elimination of the preference for the HF is consistent with the notion that WR either serves as a substitute for the rewarding value of

palatable food or as an independent behavior more favored than eating palatable foods. Additional support for this notion comes from the fact that during the two-diet choice paradigm, the amount of HF consumed was inversely correlated with the extent of WR, but the chow consumption was not. This evidence suggests that the amount of WR influences the choice between HF and chow, whereas the act of WR diminishes the overall caloric consumption.

Exercise has been shown to enhance brain leptin and insulin signaling in the CNS [17,18,25]. Leptin promotes the production of anorectic neuropeptides such as POMC and CRH while suppresses orexigenic neuropeptides such as NPY and AgRP [26–28], thus restricting any rise in food consumption. Exercise may also modulate dopamine function in the VTA or other neuro-loci, leading to a lower desire/motivation for consuming palatable foods.

Leptin effectively reduces food intake and stimulates energy expenditure in lean, young, leptin responsive rodents [29]. Although leptin regulation of energy homeostasis is largely attributed to its action in the ARC of the hypothalamus, recent evidence identified leptin function in the VTA to curb both chow and HF food consumption [10]. The effects of leptin on energy intake in the VTA are mediated by STAT3 phosphorylation [30]. Acute exercise such as two bouts of swimming increases both central insulin and leptin signaling in the hypothalamic [17]. In particular, hypothalamic STAT3 phosphorylation was elevated [17]. We recently demonstrate that unforced exercise, i.e., voluntary wheel running, also promotes STAT3 phosphorylation in the hypothalamus [18]. In obese Sprague Dawley rats chronically fed a HF diet for 5 months and provided free access to running wheels for 6 weeks, the leptin-treated and WR obese rats displayed augmented hypothalamic STAT3 signaling compared to leptin-treated sedentary obese counterparts [18]. In the present study, a short 36-hour bout of WR increased leptin-mediated STAT3 phosphorylation only in the VTA, and not in other hypothalamic or extra-hypothalamic brain regions examined. The dose of leptin administered was previously demonstrated to activate leptin signaling to maximal extent [19], thus the increased signaling appears to be the result of elevated efficacy. It is possible other factors in addition to WR may be causative to the increased signaling. For instance, the consumption of differing amounts of HF and chow food or their respective ingredients could play a role. Overall, these observations suggest a likely link between wheel running, enhanced leptin function in the VTA, and the obliteration of the preference for HF food. They also highlight the VTA as a particularly important CNS site for modulation of reward-related ingestive behavior. We speculate leptin is one factor, among many, affected by WR that plays a role in the WR-mitigation of the palatable food consumption. The mechanism by which WR could enhance leptin signaling in the VTA is unsubstantiated at the present, although it has been postulated that exercise may do so through increases in leptin receptor number that ultimately impact energy homeostasis [31].

In summary, this report demonstrates a role of WR in modulating ingestive behavior in a two-diet choice paradigm. Wheel running curtails the HF-related hyperphagia and eliminates the preference for a palatable HF diet. Our data imply that WR acts as a substitute for the behaviors related to consuming a palatable HF diet or an independent behavioral factor. Moreover, WR enhances leptin signaling specifically in the VTA, suggestive of a heightened leptin action in the VTA as one mechanism evoked by WR to temper the drive to consume palatable HF food.

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Fig 1A

Fig 1B

Fig 1C.

Fig. 1.

A: Daily food consumption in grams during four phases of the experiment 1; pre-experimental chow consumption (open circles), dietary selection between HF (dashed line, triangles) and chow (solid line, closed circles); dietary selection during WR (from days 7 to 14); and dietary selection during a final sedentary period (days 15 to 25).

B: Daily food intake in total kilocalories consumed adding both chow and HF components when both diets were available. Dotted lines divide the phases of the experiment as described in Fig. 1A.

Values represent the mean $\pm$ SE of 6 rats per group.

C: Body weight during the four phases of experiment 1 as describe in Fig. 1A

Fig 2A

Fig 2B

Fig. 2.

A: Daily food consumption in grams during four phases of the experiment 3; pre-experimental HF consumption (open squares), HF consumption during WR (solid triangles, days 0 to 7); dietary selection between HF (dashed line, open triangles) and chow (solid line, closed circles), days 7–14; and dietary selection during a final sedentary period (days 15 to 19).

B: Daily food intake in total kilocalories consumed adding both chow and HF components when both diets were available. Dotted lines divide the phases of the experiment as described in Fig. 2A.

Values represent the mean $\pm$ SE of 6 rats per group.

Fig. 3.

Correlation between daily WR and food consumption during the two-diet choice paradigm depicted in Fig. 1. The extent of WR was inversely correlated with the amount of HF food consumed ($r^2 = 0.83$, $P = 0.03$, solid squares), but not with amount of chow food consumed ($r^2 = 0.002$, $P = 0.93$, open circles).

Fig. 4.

Top: Daily food consumption in grams during dietary selection between chow (circles) and HF (squares) diet. At day zero, running wheels (dashed lines) are provided to half the rats. $P < 0.0001$ for difference between HF consumption on day 1 and day 2 between sedentary and WR groups.

$P < 0.0001$ for difference between chow consumption on day 2 between sedentary and WR groups.

Bottom: STAT3 phosphorylation in the VTA (right) and ARC (left) following vehicle (solid bars) or leptin (1 μ g, hatched bars) administration into the third cerebral ventricle on day 2 as indicated in Fig. 4, top. Rats were killed 1 hour after injection.

Values represent the mean $\pm$ SE of 6 rats per group. Results are expressed in arbitrary units per microgram of protein. P-STAT3 levels in vehicle treated sedentary rats from VTA and ARC are set to 100, and SE adjusted proportionally.

* $P = 0.01$ for difference with leptin treatment compared with corresponding vehicle administration.

** $P = 0.015$ for difference between maximum leptin stimulation in WR compared with sedentary rats and for difference with leptin treatment compared with corresponding vehicle administration.

Fig. 5.

STAT3 phosphorylation in the lateral hypothalamus (LH), dorsal medial hypothalamus (DMH), ventral medial hypothalamus (VMH) and substantia nigra (SN) following vehicle (solid bars) or leptin (1 µg, hatched bars) administration into the third cerebral ventricle on day 2 as indicated in Fig. 4, top. Rats were killed 1 hour after injection. Values represent the mean $\pm$ SE of 6 rats per group. Results are expressed in arbitrary units per microgram of protein with levels in vehicle treated sedentary rats from each brain region set to 100, and SE adjusted proportionally. *P= 0.01 for difference with leptin treatment compared with corresponding vehicle administration.

Table 1

Average daily caloric consumption in during phases of experiments 1, 2 and 3.

Diet	Activity	Time (Days)	Daily Caloric Intake (Kcal/day)
EXPERIMENT 1			
Chow	Sedentary	−4 to 0	62.9 ± 1.1
Choice (HF + Chow)	Sedentary	4 to 7	96.5 ± 4.2 [*]
Choice (HF + Chow)	WR	10 to 14	50.3 ± 3.3 [*]
Choice (HF + Chow)	Sedentary	18 to 25	87.8 ± 2.6 [*]
EXPERIMENT 2			
Chow	Sedentary	4 days	62.6 ± 1.9
Chow	WR	2 days	50.7 ± 1.7 ^{**}
EXPERIMENT 3			
HF	Sedentary	−2 to 0	85.9 ± 2.1
HF	WR	2 to 7	42.7 ± 2.8 ^{***}
Choice (HF + Chow)	WR	8 to 14	52.7 ± 2.6 ^{***}
HF	Sedentary	18 to 19	86.7 ± 3.6

Data represents mean ± SE of 6 rats in Experiments 1 and 3, and 10 rats in Experiment 2. The first 4 rows represent phases of Experiment 1 as described in Fig. 1, and the last 4 rows represent the phases of Experiment 3 as described in Fig. 2. Selected comparisons between experiment 1 and 3 are provided in the text.

* P<0.0001 for difference by one-way ANOVA. In Experiment 1, rows 2, 3 and 4 are significantly different from row 1, and row 3 is significantly different from rows 2 and 4 by Tukey post-hoc analysis (p<0.05).

** P<0.0001 for difference by t-test from chow-sedentary.

*** P<0.0001 for difference by one-way ANOVA. In Experiment 3, rows 2 and 3 are significantly different from rows 1 and 4 by Tukey post-hoc analysis (p<0.05). Rows 1 and 4 are not different from each other.