Published in final edited form as:
Physiol Behav. 2012 October 10; 107(3): 424–432. doi:10.1016/j.physbeh.2012.09.007.

Olfactory ability and object memory in three mouse models of varying body weight, metabolic hormones, and adiposity

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Abstract

Physiological and nutritional state can modify sensory ability and perception through hormone signaling. Obesity and related metabolic disorders present a chronic imbalance in hormonal signaling that could impact sensory systems. In the olfactory system, external chemical cues are transduced into electrical signals to encode information. It is becoming evident that this system can also detect internal chemical cues in the form of molecules of energy homeostasis and endocrine hormones, whereby neurons of the olfactory system are modulated to change animal behavior towards olfactory cues. We hypothesized that chronic imbalance in hormonal signaling and energy homeostasis due to obesity would thereby disrupt olfactory behaviors in mice. To test this idea, we utilized three mouse models of varying body weight, metabolic hormones, and visceral adiposity – 1) C57BL6/J mice maintained on a condensed-milk based, moderately high-fat diet (MHF) of 32% fat for 6 months as the diet-induced obesity model, 2) an obesity-resistant, lean line of mice due to a gene-targeted deletion of a voltage-dependent potassium channel (Kv1.3-null), and 3) a genetic model of obesity as a result of a gene-targeted deletion of the melanocortin 4 receptor (MC4R-null). Diet-induced obese (DIO) mice failed to find fatty-scented hidden peanut butter cracker, based solely on olfactory cues, any faster than an unscented hidden marble, initially suggesting general anosmia. However, when these DIO mice were challenged to find a sweet-scented hidden chocolate candy, they had no difficulty. Furthermore, DIO mice were able to discriminate between fatty acids that differ by a single double bond and are components of the MHF diet (linoleic and oleic acid) in a habituation-dishabituation paradigm. Obesity-resistant, Kv1.3-null mice exhibited no change in scented object retrieval when placed on the MHF-diet, nor did they perform differently than wild-type mice in parallel habituation-dishabituation paradigms of fatty food-related odor components. Genetically obese, MC4R-null mice successfully found hidden scented objects, but did so more slowly than lean, wild-type mice, in an object-dependent fashion. In habituation-dishabituation trials of general odorants, MC4R-null mice failed to

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discriminate a novel odor, but were able to distinguish two fatty acids. Object memory recognition tests for short- and long-term memory retention demonstrated that maintenance on the MHF diet did not modify ability to perform these tasks independent of whether mice became obese or were resistant to weight gain (Kv1.3-null), however, the genetically predisposed obese mice (MC4R-null) failed the long-term object memory recognition performed at 24 hours. These results demonstrate that even though both the DIO mice and genetically predisposed obese mice are obese, they vary in the degree to which they exhibit behavioral deficits in odor detection, odor discrimination, and long-term memory.

Keywords

olfactory; cognition; memory; obese; diet-induced obesity; Kv1.3

1. Introduction

Energy status and feeding state modulate olfactory ability through the effects of feeding related peptides and molecules acting at the levels of the olfactory epithelium, bulb, and cortex (reviewed by Palouzier-Paulignan et al. [1]). Insulin is secreted by the pancreas, as a result of rising levels of blood glucose in response to caloric intake, in proportion to visceral adiposity [2]. The basal and dynamic levels of insulin are therefore signals of short- and long-term energy status. Leptin is a peptide secreted by fat cells in proportion to their volume; therefore, it is considered a signal of adiposity and excess energy storage [3, 4]. Insulin and leptin are the most well studied peptides that fluctuate in concentration in relation to feeding state. They have been found to modulate the electrical activity of olfactory sensory neurons of the epithelium and of mitral cells of the olfactory bulb [5–20], as well as the organism's olfactory ability [16, 17, 21, 22]. Metabolic disorders that disrupt the normal signaling potential of these molecules also have the potential to disrupt olfactory ability.

Surprisingly, there are few studies correlating body weight or adiposity and olfactory ability in rodents, and in humans, correlations are variable. Obesity is a chronic state in which excess energy is stored as fat in adipose tissue [23–25]. Depending upon the level and duration of obesity, a variety of physiological and endocrinological changes can occur, including hyperglycemia, hyperleptinemia with associated leptin resistance, and hyperinsulimia with associated insulin resistance [26–28]. Simple obesity has been found to prevent women from typically habituating to a food cue such as an odor [29], causes an increased odor detection threshold, and decreases the ability to discriminate and identify odors in both adults and children [30, 31]. The ability to detect and identify odors in humans has been found to decrease as body mass index (BMI) increases in subjects younger than 65 years old and increases with BMI in subjects older than 65 [32]. Morbidly obese patients, those that exhibit a BMI > 45, are at a greater risk of anosmia [33], which is not reversed by significant weight loss due to gastric bypass surgery [34]. For a more complete view of the reciprocal influences of olfaction and metabolic factors see the review by Palouzier-Paulignan et al. [1].

The causes of abnormal body weight and the resulting physiological and endocrinological changes are wide ranging [35], resulting in the various changes in olfactory ability mentioned above. To truly dissect the various interactions of diet, adiposity, genetics, environment, etc. on olfactory ability, experiments in reduced controlled environments must be implemented. In this study, three well-characterized mouse models of varying body weight were used to determine the effect of body weight, adiposity, and metabolic hormones on olfactory ability and object memory. Male C57BL6/J mice (wildtype; WT) treated with a

moderately high-fat diet (MHF; 32% kcal fat; condensed-milk diet from Research Diets; D12266B; New Brunswick, NJ) were chosen for the diet-induced obesity (DIO) model [36–38]. When these mice are fed the MHF-diet for 6.5 months, they gain significantly more weight due to an increase in fat pad mass, are normoglycemic, have elevated insulin levels and are hyperleptinemic compared to mice maintained on a control chow [26, 36, 39]. Mice with a gene-targeted deletion of Kv1.3 (Kv1.3-null) are leaner than WT mice and are resistant to DIO when treated with a MHF-diet [9, 39, 40]. When crossed with a mouse model of genetic obesity, the generated MC4R/Kv1.3-null mice exhibit a significant reduction in weight gain by increasing dark phase locomotor activity and activity-dependent, mass-specific metabolism [41]. The Kv1.3 mice were chosen as the lean, obesity-resistant model. Mice with a gene-targeted deletion of the melanocortin receptor 4 (MC4R-null) were chosen for the genetic model of obesity [42–44]. MC4R-null mice are almost two times the size of their WT littermates, hyperglycemic, hyperinsulinemic, and hyperleptinemic [41, 45–48]. The results presented here are a first approximation of the correlation of body weight and adiposity on performance in olfactory-based habituation paradigms and object memory-recognition tests. The results of the manual behavioral phenotyping designs will pave the way for sensitive and automated olfactometry experiments that require more complex learning acquisition paradigms.

2. Materials and Methods

2.1. Animal Care and Mouse Lines

All animal experiments were conducted as per Florida State University Laboratory Animal Resources and American Veterinary Medical Association (AVMA)-approved methods. Food and water were provided *ad libitum* to individually housed mice in the Florida State University vivarium on a 12/12 hour light/dark cycle in accordance with institutional requirements for animal care. Kv1.3-null mice were a generous gift from Drs. Leonard Kaczmarek and Richard Flavell (Yale University, New Haven, CT) and were generated as described previously [40, 49]. The loxTB *Mc4r* mice (MC4R-null), the genetic model of obesity used in this study, were a generous gift from Dr. Joel Elmquist (University of Texas Southwestern Medical Center, Dallas, TX) and were generated as previously described [42]. Due to the well-known poor reproductive capacity of MC4R-null mice [50], MC4R heterozygous breeders were maintained and periodically crossed to establish the MC4R-null animals used in the experiments. As both male and female MC4R-null mice gain the same amount of weight [41], eight to 12 month old male and female MC4R-null mice and their wild-type littermates (WT) were used in the genetic-induced obesity experiments.

For the diet-induced obesity (DIO) model, male C57BL6/J (WT) mice were fed a moderately high-fat (MHF) diet (32% kcal fat, 51% kcal carbohydrate, 16% kcal protein; D12266B condensed-milk diet from Research Diets New Brunswick, NJ) or a control food (CF) diet (13.5% kcal fat, 58% kcal carbohydrate, 28.5% kcal protein; 5001 Purina Rodent Chow from Purina Lab Diet Feed Mill, Richmond, VA) beginning at 11 weeks of age for 6.5 months. Male Kv1.3-null mice were also treated with the DIO-regime even though they are resistant to weight gain when placed on a MHF diet [39, 40] because we wished to determine if the MHF diet, alone, could affect memory or olfactory ability. Both the WT and Kv1.3-null mice were maintained on the control 5001 Purina Rodent Chow from weaning until the beginning of the feeding treatment at ll weeks. MC4R-null and their respective WT mice were maintained on the control 5001 Purina Rodent Chow throughout their life.

2.2. Fat Pad and Serum Chemistry Analysis

To demonstrate the adiposity and serum chemistry phenotypes of each model, animals were fasted overnight and weighed. The mice were then anesthetized with isofluorane inhalation

followed by decapitation in accordance with NIH- and Florida State University Animal Care and Use Committee-approved methods. Fasting glucose, insulin, and leptin levels from trunk blood were determined as previously described [39, 41]. Briefly, an Ascensia Contour Blood Glucose Monitoring System (Bayer Healthcare, Mishawaka, IN) was used immediately following decapitation to measure blood glucose. For enzyme-linked immunosorbent assays (ELISA), serum was collected as previously described [39, 41] and stored at −20°C for later examination with a Mouse Leptin ELISA Kit (Linco Research, St. Charles, MO) and an Ultrasensitive Mouse Insulin ELISA Enzyme Immunoassay (Mercodia AB, Uppsala, Sweden) using manufacturer's protocols. All visceral fat pads, including epididymal, retroperitoneal, and mesenteric white adipose tissue, were removed from the abdominal cavity and weighed. Insulin and leptin levels were not measured for the MC4R-null mice because the extreme hyperinsulimeic and hyperleptinemic state of the MC4R knockout phenotype has been previously well established [45–48].

2.3. Experiment 1. General anosmia or buried cookie test

General anosmia tests were conducted in the last 4 hours (h) of the light phase, as previously described [9], in 24 cm wide × 47.5 cm long × 21.0 cm high rodent cages, each containing 6 cm of wood chip bedding (Harland Teklad, Madison, WI). During an experimental session, each animal was tested a total of six times. Fifty percent of the time the test used an unscented, glass marble, and 50% of the time a Ritz Bits peanut butter cracker (savory, fatty scent) or Whopper chocolate candy (sweet, chocolate scent; Publix Grocery, Tallahassee, FL), was used. Objects were hidden under the bedding along a 3 by 3 grid mapped out for the cage bottom. The center section was always used as a starting point for the animal and therefore, numbers from one to eight were written on paper and drawn to determine the position of the hidden object. For each of the six trials per experimental session, a coin was flipped to determine if the scented or unscented object was to be hidden for that particular trial. Mice were placed in the center of the experimental cage and retrieval time was measured. Each trial could last up to 600 seconds. If the object was not retrieved within 600 s, a value of 600 s was scored. For an object to be scored as retrieved, at least 5% of the object had to be visible for consistency. Between trials, each mouse was replaced in its home cage with access to food and water for two minutes. The animals were not fasted before the beginning of the experiment. Whopper experiments were performed at least one week after the peanut butter cracker trials had ended.

This behavioral paradigm, to test for general anosmia, is based upon the premise that mice will find hidden food items more quickly, based upon olfactory ability, than the random discovery of a buried, unscented object. The time taken to find the unscented marble is also a within-animal control to normalize between possible differences in locomotor activity, general curiosity, and anxiety-based digging behaviors that might increase the chances of a false positive. Statistically-different mean object retrieval times were determined using a two-way analysis of variance (ANOVA) comparing object versus diet treatment (dietary models) or object versus genotype (genetic model of obesity) at the 95% confidence level followed by a Bonferroni multiple comparisons post-hoc test.

2.4. Experiment 2. Habituation-dishabituation test

To determine if a mouse could discriminate between two structurally-different odorant pairs, such as oleic acid and linoleic acid, or peppermint and geranyl acetate, a habituation-dishabituation paradigm was employed as previously described [9, 51]. Mice were acclimated for 30 min in 16.5 cm wide × 27 cm long × 12 cm high rodent cages using standard depth wood chip bedding and without access to food or water. Testing was then initiated, whereby the mouse was habituated to "odor 1", diluted 1:100 in mineral oil and applied to a cotton swab. The cotton swab was introduced to the mouse through the top of

the testing cage and time of active investigation/smelling of the odor was recorded over a 1 minute trial period. This was repeated every 30 seconds for 7 trials. Each time, as the mouse became familiar with the odor, the investigation time decreased (habituation). On the eighth trial, "odor 2" was similarly diluted, presented and time of exploration was scored (dishabituation). For graphing purposes, all recorded times were normalized to the animal's original exploration time prior to habituation (trial 1) to minimize between-animal variance. If the animal could discriminate between the two, structurally different odors, the eighth trial would be significantly longer than that of the seventh trial. Mean investigation time of the seventh trial was compared to that of the eighth trial using a one-tailed paired *t*-test at the 95% confidence level to determine significantly-different detection of odors in a pair.

The DIO sensitive and resistant animals treated with the MHF-diet pose an interesting problem due to the chronic odor presentation of the MHF diet in the head space above the cage possibly leading to habituation or sensory specific satiety to components of the diet. The fatty acids, oleic and linoleic acid, comprise 26% and 45% of the total fat, respectively, in the MHF-diet used in this study. These fatty acids are also enriched in peanut butter [52]. Therefore, all three mouse models were screened for habituation-dishabitulation using these two fatty acids, with the anticipation that the DIO sensitive and resistant mice maintained on the MHF-diet might have difficulties with this second paradigm

2.5. Experiment 3. Short- and long-term memory test

Mice were first acclimated for 1 hour in the same type of rodent cages as in Experiment 1 without access to food and water. To prepare for testing, three plastic objects of similar size, but distinct color and shape were cleaned with 95% ethanol and rinsed with distilled water. Objects were cleaned after every trial to prevent potential odor discrimination of another animal as a means of memory recall. "Object 1" and "object 2" were used in the initial training trial, during which, the two objects were randomly placed in the front-left and front-right corners of the cage approximately 15 cm apart. The mouse was placed in the center, opposite end of the cage, away from the objects. The amount of time spent in investigation/exploration of each object was then recorded for a five-minute period, after which the objects were removed and cleaned. After the initial trial, a second trial was performed either after 1 h (to test short-term memory) or after 24 h (to test long-term memory). In this test, "object 1" (familiar) was replaced in the same front corner as previous, and then "object 3" (novel) was placed in the opposite front corner. The investigation/exploration time was again recorded for each object during a 5 minute trial period. If a mouse remembers the familiar object, the premise of the test is that it will spend more time investigating the novel object, "object 3", in the second trial. If the mouse does not recognize the familiar object, "object 1", it will investigate each object equally.

3. Results

3.1. Body weight, serum chemistry, and visceral adiposity in three mouse models

Final body weights of mice maintained on the MHF diet were recorded prior to behavioral phenotyping (Experiments 1–3). DIO was evident in WT mice after 6.5 months on the 32% fat diet as compared with mice maintained on the CF diet that contained only 13.5% fat (45.9 ± 0.9 g, MHF versus 31.9 ± 0.8 g, CF). The Kv1.3-null mice, on the other hand, were highly resistant to the same dietary regime (30.74 ± 1.6 g, MHF versus 26.9 ± 0.8 g, CF). A two-way analysis of variance (ANOVA) across genotype and diet demonstrated significantly-different basal body weight on CF diets across genotype and significantly-different body weight attributed to diet in WT (43.9 % increase) and Kv1.3-null (14.3 % increase) mice (Fig. 1, left). The diet-induced increase in weight of the Kv1.3-null mice, however, only brought its weight up to that of WT-CF mice. Upon completion of behavioral

experiments, a subset of the CF and MHF-diet treated animals were fasted overnight and blood glucose, serum insulin and leptin, and visceral fat mass were measured and are reported in Table 1. MHF-diet treatment significantly increased fasting insulin and leptin levels as well as visceral adiposity in the DIO sensitive C57BL6/J mice without modifying fasting glucose levels or resulting in hyperinsulinemia as has been previously shown [26, 36, 39, 40].

Genetically-induced obesity was also documented in the MC4R-null mice prior to behavioral phenotyping (Experiments 1–3). Eight month old MC4R-null mice were confirmed to be significantly heavier than their aged-matched WT counterparts (51.9 ± 2.13 g, MC4R-null versus 29.0 ± 1.34 g, WT; Fig. 1, right; Student's *t*-test, 95% confidence interval). It is well established that by this age and body weight, the MC4R-null mouse is hyperglycemic, hyperinsulinimic, and hyperleptinemic with significantly increased adiposity [45–48] (for review see [43, 44]). However, to confirm the obese phenotype and ease comparison between the models, fasting blood glucose and visceral fat mass are reported in Table 1 for a subset of the animals used in the behavioral trials.

3.2. Experiment 1

In the first set of anosmia tests, the time taken to find an unscented, glass marble versus a Ritz Bits peanut butter cracker (savory, fatty scent) was compared for WT and Kv1.3-null mice treated with either a CF- or MHF-diet for six months. WT mice maintained on the MHF diet did not exhibit a significantly faster retrieval time to uncover the cracker versus the marble, whereas those maintained on CF diet had no difficulties retrieving the food item faster (Fig. 2A; two-way ANOVA). The follow-up test also demonstrated that WT mice on the MHF diet retrieved the cracker significantly slower than did mice on the CF diet. For the Kv1.3-null animals, diet had no effect on retrieval time; animals maintained on CF- or MHF-diet for six months were equally efficient in finding the hidden food item significantly faster than that of the marble (Fig. 2B; two-way ANOVA). When an identical test was applied to the genetically-obese mice, both WT and MC4R-null mice had the capacity to uncover the cracker significantly faster than that of the unscented marble (Fig. 2C; two-way ANOVA). The follow-up test also demonstrated that MC4R-null mice retrieved the cracker significantly slower than did WT mice. These data suggest that DIO perturbs the ability to detect fatty scents whereas mice made fat through genetic means and not consumption of a fat diet, retain the ability to detect the fatty scent. To test this idea, we changed the hidden food item to one that would generate a sweet, chocolate scent (Whopper). In rescreening the DIO obese mice, interestingly, both WT and Kv1.3-null animals were unaffected by diet and both genotypes found the hidden chocolate Whopper significantly faster than that of the marble (Fig. 2D–E; two-way ANOVA). Moreover, the genetically-obese mice could also find the hidden chocolate Whopper significantly faster than that of the marble, albeit performing a slower retrieval in both WT and MC4R-null mice for the chocolate (Figure 2F) versus the cracker food (Fig. 2C) item.

3.3. Experiment 2

The fatty acids, oleic and linoleic acid, comprise 26% and 45% of the total fat in the MHF diet used in this study, respectively. Linoleic and oleic acid differ by a single double bond and were indistinguishable by the investigator. To determine if the mice treated with a MHF diet could detect and discriminate between the very similar dietary fatty acids, linoleic and oleic acid, a habituation-dishabituation odorant paradigm was employed [9, 51]. WT and Kv1.3-null mice, independent of diet treatment and body weight, could readily distinguish between the very similar fatty acids (Fig. 3A; one-tailed paired *t*-test). MC4R-null mice were also able to distinguish between the oleic and linoleic acids (Fig. 3B; one-tailed paired *t*-test). On the other hand, these genetically-obese mice failed an identical habituation-

dishabituation odorant paradigm when the odorant pair to be discriminated was comparatively much easier. Here, the habituated odorant was peppermint extract whereas the dishabituation trial used geranyl acetate. WT mice had no difficulty discriminating these two odorants, whereas investigation time for the MC4R-null mice was not significantly different between the seventh and eighth trials, signifying lack of the ability to discriminate between the two odors (Fig. 3C; one-tailed paired *t*-test).

3.4. Experiment 3

General anosmia and odor habituation-dishabituation trials do not require memory, however, olfactory tests that screen for odor detection threshold (i.e. two-choice paradigm [9]) or those that incorporate odorant adaptation or automation (i.e. olfactometry [53]) require both learning and memory consolidation on both a short- and long-term basis. Because DIO in rodents has previously been shown to affect memory and cognitive function [54–56], short- and long-term memory were assessed by object recognition testing as previously described [9, 57, 58]. DIO-sensitive WT (Fig. 4A, B) and DIO-resistant Kv1.3-null mice (Fig. 4C, D) increased exploratory time to a novel object (object 3) following either a 1 or 24 h test interval between the presentation of two initial objects to establish familiarity (objects 1 and 2) (significantly different by Arc-Sine transformation for percentage data followed by a Student's *t*-test, $P < 0.05$). Maintenance on a MHF diet had no effect on object memory recognition across genotype (Fig. 4B, 4D, respectively). Interestingly, MC4R-null mice when subjected to the same paradigm, failed to increase exploratory time to a novel object following a 24 h test interval between the presentation of two initial objects to establish familiarity (objects 1 and 2) (not significantly different by Arc-Sine transformation for percentage data followed by a Student's *t*-test, $P < 0.05$). These data suggest that genetically-obese mice have cognitive problems in long-term object memory that are not present in short-term tests nor are they observed in mice fed a MHF diet.

4. Discussion

While cheap, readily available, calorically-dense foods and decreased physical activity are to blame for a large portion of the obesity epidemic, other factors have begun to draw the attention of the scientific community as contributing factors, such as genetic and epigenetic factors, increasing maternal age, increased birthrates in the obese community due to medical intervention, sleep deprivation, endocrine disruptors, pharmaceutical side effects, and stabilization of ambient temperatures [35]. With so many factors contributing to obesity in the human population, it is easy to see the difficulty in investigating how obesity, and all it encompasses, affects any one system. That is why in this study, three different mouse models, a diet-induced obesity (DIO) model, an obesity-resistant model and a model of mono-genetic obesity were used to investigate the effects of obesity and diet on sensory function, or olfaction.

DIO sensitive, C57BL6/J mice (WT) and obesity-resistant, Kv1.3-null mice, were fed either a CF- or MHF-diet for 6.5 months. Because body weight significantly increased by 20 g in the WT mice challenged with the MHF diet and remained relatively unchanged in the Kv1.3-null mice, we had the ability to investigate the effect of the diet separate from that of increased adiposity and metabolic hormones on olfactory ability. A genetic model of obesity, the MC4R-null mouse, was also used to evaluate change in olfactory ability, between the ages of eight to 12 months when body weight was also increased by nearly 30 g, but not attributed to increased dietary fat.

By using a general test for anosmia, sometimes called the buried cookie test, DIO sensitive, WT mice made obese through maintenance on the MHF diet, failed to find a buried peanut butter cracker (savory, fatty scent) significantly faster than an unscented marble, whereas

Kv1.3-null mice, maintained on the same MHF diet (but obesity resistant), had no difficulty in retrieving the hidden food object. This suggested that the elevated adiposity and resulting increase in serum leptin and insulin were the principle variables contributing to anosmia rather than the fat in the diet. On further inspection, however, the retrieval time to find a hidden Whopper chocolate candy (sweet, chocolaty scent) was significantly faster than that for a marble in both WT and Kv1.3-null mice, and was independent of diet. Because linoleic and oleic acids are components of peanut butter and the MHF chow, and can be well discriminated by WT mice following DIO, it is unlikely that lack of rapid retrieval of a peanut butter scented object is attributed to habituation to these specific fatty odors following chronic odor presentation of the MHF diet in the head space above the cage.

In humans, sensory-specific satiety is a phenomenon in which a food item is eaten to the point of satiety resulting in the reduction of the pleasantness of that food item [59]. Alliesthesia is a change in the pleasantness (liking) and appetence (desirability) of a sensation due to changes in internal state, such as hormonal changes before and after a meal. Interestingly, Plailly et al. found that appetence is decreased more for fatty food odors than other food odors after a meal in humans [60]. It is possible that as the mice had *ad libitum* access to the MHF chow, the animals were experiencing sensory-specific satiety or negative alliesthesia to fatty scented food objects such as the peanut butter cracker. The Whopper chocolate candy, however, was a completely new odor and the mice were therefore motivated to investigate the novel food odor. If motivation played a role in the responses of the WT mice following DIO, the Kv1.3-null mice did not appear to have demonstrated these motivational differences. The reason that MHF fed, Kv1.3-null mice still retrieve the fatty scented object is not understood. Future experiments should take advantage of utilizing alternative means of motivation other than food, such as water deprivation [53] to prevent motivational interference.

The obesity-resistant Kv1.3-null mice have previously been shown to have a significantly lower odor detection threshold and greater odor discrimination abilities, for most, but not all odor pairs tested, than their WT counterparts [9]. When these mice were tested with oleic and linoleic acid in the habituation-dishabituation paradigm, they had no problem distinguishing between the two fatty acids independent of diet regime, but also performed no better than WT mice. Oleic and linoleic acids were not tested in these mice previously and now prove to be another pair, like C7/C9 and C7/C10 alcohols [9], for which the Kv1.3-null mice do not discriminate more easily than WT.

While MC4R message has been reported in the olfactory tubercle and lateral olfactory tracts and reporter transgene expression under the control of the MC4R promoter has also been localized to these regions [61, 62], there are no reports of MC4R expression in the olfactory bulb nor have there been published studies of the olfactory ability of MC4R-null mice. MC4R-null mice took twice as long as WT mice to find the hidden peanut butter cracker in the general anosmia test, but were not found to be anosmic. In fact, when the paradigm was switched to chocolate, they could find this scented object on the same time scale as did WT mice. An explanation of why both WT and MC4R-null mice took longer to find the chocolate over that of the peanut butter cracker is not known, nonetheless they could retrieve the scented object more quicky. Habituation-dishabituation trials incorporating general odorants demonstrated the inability of the MC4R-null mice to discriminate. When screened for discrimination across fatty acids, however, they exhibited no difficulty in discerning between oleic and linoleic acid. This is very interesting in light of the results of Getchell et al., [21] that showed that obese, leptin deficient ob/ob mice and leptin receptor deficient db/db mice were able to find a hidden peanut butter cracker in the general anosmia test 10x faster than WT mice. When leptin was administered to the leptin deficient ob/ob mice, the food finding time increased back to WT levels. Leptin administration to normal weight,

fasted rats has also been found to decrease olfactory sensitivity [16]. MC4R signaling is downstream of the leptin receptor expressing POMC neurons of the hypothalamus known to regulate energy balance [63]. Leptin receptors are also expressed in the olfactory epithelium [18, 21, 64] and the olfactory bulb [65], but have never been linked with MC4R signaling there. Despite reported leptin resistance in the MC4R-null mice, the high circulating peripheral leptin levels could be acting at the level of the olfactory epithelium, which is outside the blood brain barrier, or at the level of the OB which has not been tested for leptin resistance, to decrease olfactory sensitivity resulting in poor performance on the discrimination test and the slower retrieval time in the anosmia test.

MC4R-null mice are also hyperglycemic, hyperinsulinemic and insulin resistant (reviewed by Butler and Cone [44]). Acute application of both leptin and insulin increase the spontaneous activity of olfactory receptor neurons and decrease odor-evoked responses in olfactory epithelium slice recordings from rats [12]. Electroolfactogram responses to isoamyl acetate were also reduced in response to insulin and leptin. An effect which, the authors suggest, is a result of an over all decrease in the signal to noise ratio of the system, ultimately resulting in a decrease in sensitivity [12]. It has also been shown that insulin modulates the pattern of intermittent action potential clusters generated by mitral cells of the olfactory bulb, which are responsible for contribution to the olfactory code [10]. Elevation of insulin attributed by DIO or via intranasal delivery, causes insulin resistance in the olfactory bulb, disrupts mitral cell evoked firing patterns, and alters protein-protein interactions in known olfactory signaling cascades [10, 22].

General anosmia screening and habituation-dishabituation paradigms do not require consolidation of memory whereas odor threshold discrimination (i.e. two choice paradigm[9]) or operant conditioning (i.e. olfactometry [53]) necessitates a phase of learning acquisition as well as long-term memory. High-fat diets and obesity have previously been shown to impair learning and memory [54, 56, 66], therefore our results comparing object memory recognition across the three mouse models are of particular consequence to olfactory behavioral phenotyping. While consumption of MHF diet did not interfere with the short- or long-term object recognition tasks, gene-targeted deletion of MC4R, however, may have impacted the ability of the MC4R-null mice to successfully pass the 24 h, long-term object memory recognition task, not the obesity itself. MC4R is not only expressed in the hypothalamus, but in areas important for learning and memory as well, such as the hippocampus and amygdala [62]. This receptor has previously been shown to be involved in memory consolidation, reconsolidation and procedural memory learning [67–69]. The MC4R-null mouse may therefore not be amendable to advanced sensory discrimination tests that employ advanced cognition or long-term memory.

Body weight and adiposity are influenced at multiple levels by an individual’s genetic makeup and environment. Abnormal body weight due to changes in adiposity, such as in obesity or anorexia, result in concomitant changes in blood chemistry and metabolism affecting multiple systems to varying degrees. These cause and effect cascades make it impossible to make accurate inferences about the effect of “generic obesity” on a particular system because depending on the cause or combination of contributing factors the resulting phenotype (hormones, metabolism, motivation, and preference) will be very different. In fact, the studies presented here demonstrate that behavioral performance of mice in olfactory sensory tests that do not require learning or memory are differentially modified in two mouse models of obesity. Moreover, not all mouse models can be used in advanced sensory discrimination tests due to deficits in long-term object memory that may impede interpretation or acquired learning in the animal. Finally, the answer to the question, “ Do fat mice smell, and if so, do they remember it?” depends upon the mouse model employed – our

data demonstrate that not all mouse models of obesity result in the same olfactory and memory changes.

Acknowledgments

We would like to thank Mr. Michael Henderson, Mr. Robert Daly, and Ms. Stephanie Zych for routine animal husbandry during the course of our studies. Our work was supported by grants from the US National Institutes of Health (NIH), R01DC003387 (to DF) and T32 DC00044 (to KT), both from the National Institutes of Deafness and Communication Disorders (NIDCD). We thank Bayer Healthcare for their generous donation of glucose testing strips used with their Ascensia Contour Blood Glucose Monitoring System.

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Highlights

- Diet-induced and MC4R-dependent obesity slow odor-dependent food finding time.
- Mice with MC4R-dependent obesity have a reduced ability to discriminate some odors.
- Moderately high-fat diet or correlated obesity does not affect object memory in mice.
- Mice with MC4R-dependent obesity exhibit deficits in long-term object memory.

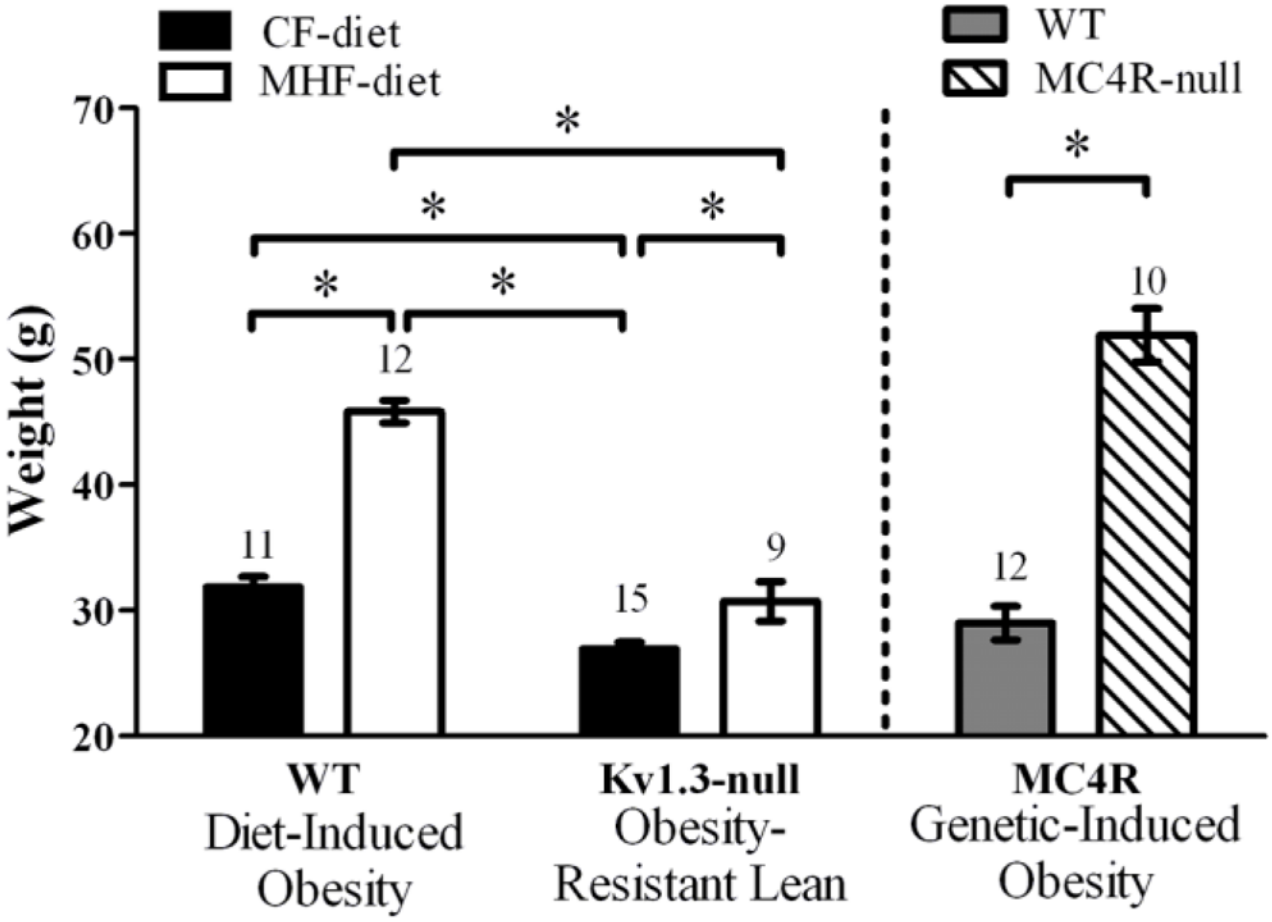

Fig. 1.

Body weight comparison across three mouse models – 1) diet-induced obesity, 2) obesity-resistant lean mice, and 3) genetic-induced obesity. (LEFT) Bar graph of the mean (± standard error of the mean; SEM) body weight of wild-type (WT) and Kv1.3−/− (Kv1.3-null) mice maintained on either a control Purina chow (CF; 13.5% fat) or a moderately high-fat (MHF; 32% fat) diet for 6.5 months. * = significantly-different by two-way analysis of variance (ANOVA) with a Bonferroni multiple comparisons post-hoc test, $P < 0.05$. (RIGHT) Bar graph of the mean (± SEM) body weight of 8–12 month old wild-type (WT) and MC4R-null mice maintained on CF since weaning. * = significantly-different by Student's *t*-test, $P < 0.05$ for MC4R-null vs WT. Number of mice per treatment group as indicated.

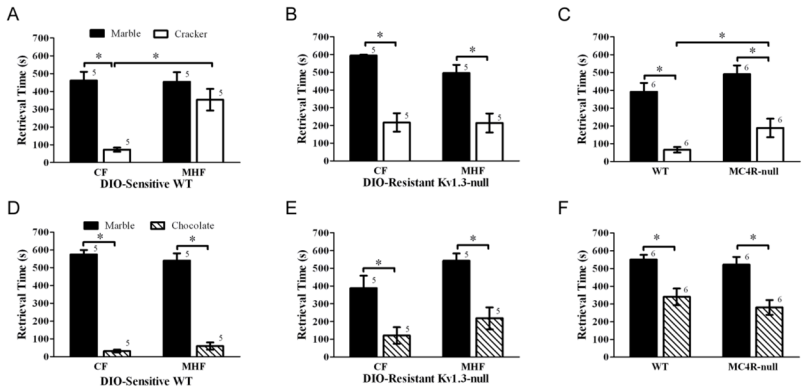

Fig. 2.

General anosmia testing in three models of varying body weight, metabolic hormones and visceral adiposity. Bar graphs of the mean (± SEM) retrieval time to find a hidden, (A–C) fatty-scented object (cracker, open bar) or (D–F) sweet-scented object (chocolate, hatched bar) versus an unscented object (marble, black bar) for (A, D) diet induced obesity (DIO) prone wild-type (WT) and (B, E) DIO resistant Kv1.3-null mice maintained on either a control (CF) or moderately high-fat (MHF) diet for 6.5 months or, (C, F) MC4R WT (WT) versus MC4R-null (MC4R-null) mice maintained on CF from weaning to 8–12 months of age. * = significantly-different by two-way ANOVA within genotype across diet and object, $P < 0.05$, Bonferroni multiple comparisons post-hoc test. Number of trials for various treatment groups as indicated.

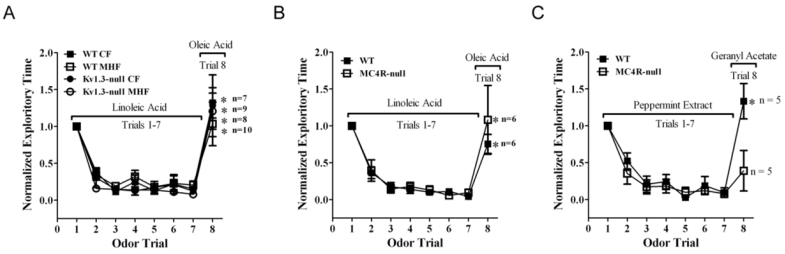

Fig. 3.

Habituation-dishabituation testing for odorant discrimination in three models of varying body weight, metabolic hormones and visceral adiposity. (A) Line graph of the normalized exploratory time for a mouse in response to the repeated presentation of the habituating odor (Linoieic Acid; Trials 1–7) followed by the dishabituating odor (Oleic Acid; Trial 8). CF = control food, MHF = moderately high-fat diet. (B) Same as in A, but comparing habituation-dishabituation ability between MC4R WT and MC4R-null mice. (C) Same as in B, but using Peppermint extract for the habituating odor (Trials 1–7) and Genanyl Acetate for the dishabituating odor (Trial 8). (A–C) Same dietary duration and ages as in Fig. 2. * = significantly-different by one-tailed paired *t*-test comparing time of exploration at Trial 7 versus that at Trial 8. Number of mice tested for various treatment groups as indicated.

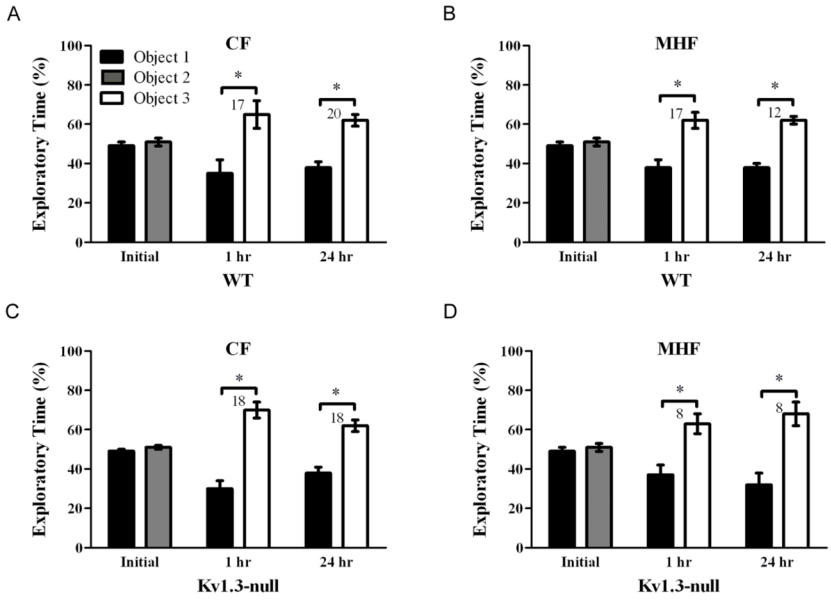

Fig. 4.

Object memory recognition for short- and long-term memory in mice maintained on a control or moderately high-fat diet. Bar graph comparing the percentage of time spent exploring two different objects (Object 1, Object 2) during an initial exploratory time, and then the change in percentage of exploration following representation of the familiar object (Object 1, dark bar) versus that of a novel object (Object 3, open bar) in the same group of animals 1 h (1 hr) or 24 h (24 hr) later. * = significantly-different by Arc-Sine transformation of percentile data followed by a Student's t-test, $P < 0.05$. Notations and dietary duration as in Fig. 1.

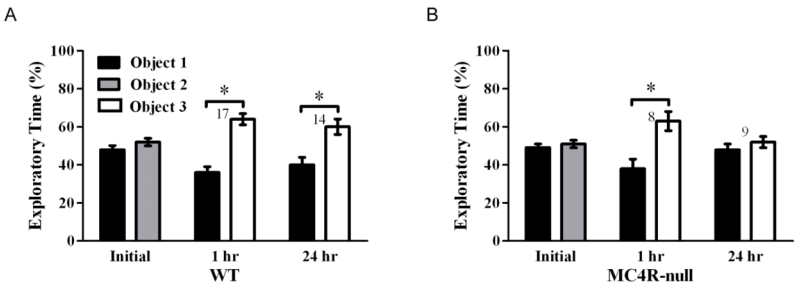

Fig. 5.

Object memory recognition for short- and long-term memory in a mouse model of genetic obesity. Experimentally the same as in Fig 4, but comparing object memory recognition for WT versus genetically-obese mice (MC4R-null) maintained on a control diet. Notations and ages as in Fig. 1. Same statistical design as in Fig. 4.

Table 1

Fasting serum chemistry and visceral fat mass. Visceral fat mass and dark-phase fasted glucose, insulin, and leptin levels of the DIO sensitive (C57Bl6/J, WT) and DIO resistant (Kv1.3-null) mice treated for 26 weeks with either a CF- or MHF-diet.

	DIO Sensitive C57BL6/J WT	DIO Resistant Lean Kv1.3-null	Genetic-Induced Obesity	
Fasting Glucose (mg/dl)				
CF-diet	97.6 ± 7.5 (5)	95.4 ± 5.9 (5)	WT	103 ± 16 (6)
MHF-diet	105 ± 5.6 (5)	71.3 ± 12.8 (3)	MC4R-null	148.4 ± 28 (5)
Fasting Insulin (ng/ml)				
CF-diet	0.46 ± 0.05 (5)	0.39 ± 0.05 (5)	WT	1.3–4.2#
MHF-diet	1.02 ± 0.25 (5)*	0.62 ± 0.17 (4)	MC4R-null	16.3–75.4#
Fasting Leptin (ng/ml)				
CF-diet	3.59 ± 1.41 (4)	1.10 ± 0.17 (4)	WT	13.5–14#
MHF-diet	27.13 ± 2.3 (4)*	6.43 ± 3.14 (4)	MC4R-null	75.7–87.2#
Visceral Fat Mass (g)				
CF-diet	1.48 ± 0.22 (5)	0.63 ± 0.18 (5)	WT	1.34 ± 0.32 (6)
MHF-diet	4.66 ± 0.35 (5)*	0.98 ± 0.29 (5)	MC4R-null	4.99 ± 1.1* (5)

For the DIO sensitive and resistant mice, * = significantly-different by two-way ANOVA across genotype and diet with a Bonferroni multiple comparisons post-hoc test, $P < 0.05$.

For the genetic-induced obesity model, * = significantly-different by Student's *t*-test, $P < 0.05$ for MC4R-null vs WT.

# indicates range of unfasted serum levels from 9–10 month old female and male WT and MC4R-null mice taken from Marie *et al.,* 2000 [46]