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Basal ganglia dysfunction contributes to physical inactivity in obesity

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Summary

Obesity is associated with physical inactivity, which exacerbates the health consequences of weight gain. However, the mechanisms that mediate this association are unknown. We hypothesized that deficits in dopamine signaling contribute to physical inactivity in obesity. To investigate this, we quantified multiple aspects of dopamine signaling in lean and obese mice. We found that D2-type receptor (D2R) binding in the striatum, but not D1-type receptor binding or dopamine levels, was reduced in obese mice. Genetically removing D2Rs from striatal medium

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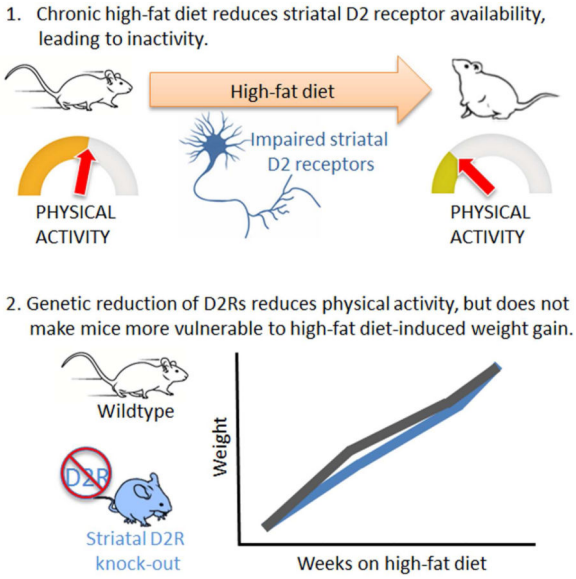
spiny neurons was sufficient to reduce motor activity in lean mice, while restoring G_i signaling in these neurons increased activity in obese mice. Surprisingly, while mice with low D2Rs were less active, they were not more vulnerable to diet-induced weight gain than control mice. We conclude that deficits in striatal D2R signaling contribute to physical inactivity in obesity, but inactivity is more a consequence than a cause of obesity.

Graphical Abstract



Introduction

Obesity is associated with physical inactivity (Brownson et al., 2005; Ekkekakis et al., 2016), which compounds the negative health effects of type-II diabetes and cardiovascular disease (de Rezende et al., 2014; Sharma et al., 2015). The mechanisms that underlie this association are not known, a fact reflected in the lack of effective interventions for altering physical activity levels in populations with obesity (Ekkekakis et al., 2016). Interestingly, obesity has been associated with alterations in striatal dopamine (DA) signaling, which has led to hypotheses of reward dysfunction in obesity (Blum et al., 2011; Kenny, 2011; Volkow and Wise, 2005). Although striatal DA is strongly linked to motor output, few studies have investigated how diet-induced dopaminergic alterations might contribute to physical inactivity. We hypothesize that striatal DA signaling is impaired in obesity, and that this contributes to physical inactivity. Understanding the biological causes of physical inactivity may lead to effective interventions for increasing activity, and thereby improving health, in individuals with obesity.

Striatal DA is critically involved in motor control. This is evident in motor disorders such as Parkinson’s disease, which is characterized by the death of dopaminergic neurons in the midbrain and resulting loss of striatal DA (Hornykiewicz, 2010). The two populations of striatal projection neurons modulated by DA are known as the direct and indirect pathway medium spiny neurons (dMSNs and iMSNs) (Alexander and Crutcher, 1990; DeLong, 1990;

Gerfen et al., 1990). dMSNs express the G_s-coupled D1 receptor (D1R) and project to the substantia nigra and internal segment of the globus pallidus, whereas iMSNs express the G_i-coupled D2R and project to the external segment of the globus pallidus (GPe) (Gerfen et al., 1990; Le Moine and Bloch, 1995; Levey et al., 1993). Genetic elimination of D2Rs from iMSNs, or optogenetic stimulation of iMSNs, is sufficient to reduce movement (Kravitz et al., 2010; Lemos et al., 2016). Based on links between D2R dysfunction and obesity, we hypothesized that obese animals have altered iMSN output, resulting in physical inactivity.

Here, we examined multiple aspects of DA signaling in lean and diet-induced obese mice. D2R binding was reduced in obese mice whereas D1R binding and extracellular DA levels remained unchanged. Obese mice also exhibited disruptions in striatal firing, and had reduced movement. Genetically eliminating D2Rs from iMSNs reduced activity in lean mice, whereas restoring G_i signaling in iMSNs increased activity in obese mice. These results establish that D2R signaling in iMSNs can bi-directionally modulate physical activity. We then asked whether mice with low D2R signaling were more vulnerable to weight gain on a high fat diet, due to their low activity.

To do this, we examined weight gain with respect to natural variation in D2R binding among mice, as well as in mice with genetic elimination of striatal D2Rs. While mice with low levels of D2Rs had low levels of physical activity, they gained weight at the same rate as mice with intact D2Rs. This argues against a strong causal relationship between physical activity and weight gain. We conclude that impairments in D2R signaling contribute to physical inactivity in obesity, but that inactivity does not necessarily lead to weight gain.

Results

Diet-induced obesity was associated with physical inactivity

C57BL6/J male mice (3–4 months) were fed either standard chow (lean, $n = 8$) or high-fat diet (obese, $n = 8$) for 18 weeks (Figure S1a). Beginning at week 2 and persisting through week 18, obese mice had significantly higher body weight and fat mass than lean mice ($p < 0.0001$; Figure 1a, S1b). Lean mass was not significantly altered (Figure S1c). We measured activity levels in an open field every 2 weeks for 18 weeks (Ethovision, Noldus Information Technologies). Obese mice had lower activity than lean mice beginning at week 4 and persisting through week 18 ($p < 0.0001$; Figures 1b–c). At week 18, obese mice spent less time moving ($p = 0.005$), had fewer movements ($p = 0.0003$), and had slower speeds while moving ($p = 0.0002$, Figure 1d) relative to lean mice. Rearing and grooming were not significantly altered (Figure 1d). Obese mice also ran less than lean mice when given access to home-cage running wheels ($p = 0.0005$; Figure 1e). We tested whether movement deficits correlated with weight gain in the obese group. Although weight gain was correlated with caloric intake of high-fat diet (Figure 1f), it was not correlated with movement levels in an open field or with energy expended during the high-fat diet period (Figures 1g, h). Interestingly, these same correlations held when we examined food intake in the first week of the experiment (Figures 1i–k), indicating that initial levels of high-fat diet intake (but not movement or energy expenditure) was predictive of later weight gain.

Obesity was associated with reductions in dopamine D2R binding

To identify mechanisms underlying physical inactivity, we quantified multiple aspects of DA signaling in lean and obese mice. Consistent with prior reports in rodents, D2R-like receptor binding (via autoradiography with ^{3}H-spiperone, hence-forth termed D2R-binding) was lower in obese mice relative to lean mice ($p < 0.0001$; Figures 2a–b), a finding that was significant in all three striatal subdivisions (dorsomedial: $p = 0.004$; dorsolateral: $p < 0.0001$; ventral: $p < 0.001$; Figures S2a, b). However, D2R binding was not correlated with body fat in the lean or obese group ($p > 0.55$ for both, Figure 2c) suggesting that although D2R binding and fat storage are both altered by chronic high-fat diet, these variables may not be causally related to one another.

We attempted to identify the mechanism underlying obesity-mediated reduction in D2R binding. To do this, we looked for differences in *Drd2* mRNA (via *in situ* hybridization) and found it unchanged in all three striatal subdivisions (dorsomedial: $p = 0.92$; dorsolateral: $p = 0.90$; ventral: $p = 0.34$; Figure S2c). We performed western blots to quantify total D2R protein levels, and noted no change in either the 50kDa or 70kDa bands, thought to represent different glycosylation states of the D2R (both $p > 0.95$, Figures S2d–e) (Johnson and Kenny, 2010). Finally, we evaluated markers of metabolic dysfunction in lean and obese mice to see if they might relate to the decrease in D2Rs as previously reported (Dunn et al., 2012). Obese mice had higher fasting cholesterol ($p < 0.0001$), leptin ($p < 0.0001$), glucose ($p = 0.0002$), insulin ($p = 0.001$), and resistance-based homeostatic model assessment (HOMA-IR; $p < 0.001$), but not triglycerides or free fatty acids (Figures S1d–j). However, none of these factors correlated with D2R binding in obese mice (data not shown).

D1R-like binding (via autoradiography with ^{3}H-SCH23390, hence-forth termed D1R binding) did not differ between obese and lean mice ($p = 0.20$; Figure 2d). There were also no differences in striatal DA content, measured via high performance liquid chromatography (HPLC) of striatal tissue punches ($p = 0.41$; Figure 2e), or tyrosine hydroxylase immuno-labeling ($p = 0.64$; Figure 2f). In light of multiple reports of differences in basal DA in obese mice (Carlin et al., 2013; Davis et al., 2008; Vucetic et al., 2012; Wang et al., 2014), we further explored this point using no-net-flux microdialysis (new mice, $n = 6$ per group). We again observed no differences in extracellular DA ($p = 0.99$) or either of its two metabolites, DOPAC ($p = 0.85$) and HVA ($p = 0.68$, Figure S3) with this method, indicating that obesity was not associated with reductions in extracellular DA tone in these experiments.

Movement-related striatal firing was disrupted in obese mice

We performed *in vivo* electrophysiology to examine how reduced striatal D2R binding might alter striatal neuronal output, and thereby contribute to reductions in movement. We recorded from the dorsomedial striatum of lean and obese mice ($n = 3$ mice per group, histology in Figure 3f). Although obese mice moved less overall, the velocity of executed movements did not differ between these groups ($p = 0.55$; Figure 3a), allowing us to compare movement-related firing between lean and obese mice. Basal multi-unit spiking rates did not differ between the lean and obese mice (lean 2.1 ± 0.4Hz, obese: 2.0 ± 0.7Hz, $p = 0.93$). However, the prevalence of movement-activated units (Figure 3b) was markedly lower in obese mice ($p < 0.0001$; Figure 3c). This did not depend on our statistical definition

of "movement-activated" units, as we also observed reduced spiking around movements in the average response of all recorded units in obese vs lean mice (interaction by ANOVA, $p < 0.0002$; Figure 3d–e). We conclude that total spiking rate in the striatum did not differ, but the organization of spikes around movement was disrupted in obese mice.

Inhibition of iMSN output restored activity levels in obese mice

To test whether reducing the output of iMSNs could increase movement in obese mice, we used a Cre-recombinase (Cre) dependent strategy to express an inhibitory G_i-coupled modified kappa opioid receptor designer receptor exclusively activated by designer drugs (KOR-DREADD) in iMSNs of obese mice (Figure 4a). Although the Adenosine$_{2A}$-receptor Cre (A2A-Cre) mouse has been previously validated with immunostaining to demonstrate that Cre expression is specific to striatal iMSNs (Cui et al., 2013; Lemos et al., 2016), we performed an additional validation of this line with double fluorescent *in situ* hybridization. Nearly all neurons (98.7 ± 0.6% of 1301 counted neurons) expressed both *Cre* and *Drd2* mRNA, whereas very few (1.3 ± 0.6%) expressed either *Cre* or *Drd2* mRNA, but not both, confirming that the A2A-Cre line faithfully targets iMSNs (Figure S4).

Injections of the KOR-DREADD agonist salvinorin-B (SalB) increased the distance traveled by obese mice expressing the KOR-DREADD ($p = 0.02$; Figure 4b). SalB also increased the frequency of rearing ($p = 0.02$; Figure 4f), and caused a trend towards an increase in frequency ($t_{(7)} = 1.64$, $p = 0.12$), but not duration or speed, of movement (Figures 4c–e). Injections of SalB also increased movement in lean mice ($p = 0.01$; Figure 4h), but not in wildtype mice that did not express the KOR-DREADD ($p = 0.73$; Figure 4i). We conclude that reducing the output of iMSNs is sufficient to increase movement levels of both lean and obese animals.

Low D2R levels do not predispose animals to future weight gain

Finally, we examined whether pre-existing differences in D2R signaling might predispose individual mice to diet-induced obesity. To address this question, we performed micro positron emission topography (microPET) with ^{18}F-fallypride to determine baseline D2R availability prior to high-fat diet exposure (Figure 5a). We noted a high level of variance in D2R binding potential among mice, as others have shown (Constantinescu et al., 2011). Individual differences in D2R availability were positively correlated with movement in the open field ($p = 0.045$; Figure 5b), consistent with the role of D2Rs in movement. Following microPET scanning, animals were maintained on a high-fat diet for 18 weeks, to test whether mice with low D2Rs would be more vulnerable to diet-induced weight gain. Surprisingly, we found a trend towards a *positive* relationship between initial D2R availability and weight gain across this experiment ($p = 0.10$; Figure 5c). Although this correlation was not significant, it argues against the hypothesis that low D2R availability or low physical inactivity makes animals more vulnerable to weight gain. This was also consistent with our findings that neither basal open field activity, nor open field activity across the entire experiment, correlated with weight gain (Figures 1f–k).

To further explore the relationship between pre-existing differences in activity levels and weight gain, we took advantage of a genetic mouse model with targeted deletion of the *Drd2*

gene from iMSNs (iMSN-*Drd2*-KO), but preserved expression in other cell types (Dobbs et al., 2016; Lemos et al., 2016). As previously reported, iMSN-*Drd2*-KO mice moved less than littermate controls in an open field ($p = 0.02$; Figure 5e) and on home cage running wheels ($p = 0.01$; Figure 5f). Consistent with the above experiments, iMSN-*Drd2*-KO mice did not gain more weight than their littermate controls when placed on a high-fat diet ($p = 0.23$; Figure 5g). To examine their energy utilization more closely, we performed indirect calorimetry experiments to compare iMSN-*Drd2*-KO mice to littermate controls. We did not detect significant differences in energy intake ($p = 0.60$), energy expenditure ($p = 0.47$), or respiratory exchange ratio (RER; ratio of CO_2 production to O_2 consumption [VCO_2 / VO_2], $p = 0.17$) between iMSN-Drd2-KO mice and their littermate controls, indicating that the reductions in movement of the IMSN-Drd2-KO mice did not translate into changes in energy utilization (Figure 5h–j). Finally, we explored the extent to which smaller reductions in striatal D2R (such as those observed in our obese mice) could regulate movement and weight gain. To do this, we used a mouse line that results in a 30–40 percent decrease in striatal Drd2R RNA (iMSN-Drd2-Het) (Lemos et al., 2016). These mice also exhibited reduced movement, demonstrating that a partial knock-down of the D2R is sufficient to produce motor deficits ($p = 0.04$; Figure S5a). Similar to iMSN-Drd2-KO mice, iMSN-Drd2-het mice were not more susceptible to high-fat diet induced weight gain ($p = 0.89$; Figure S5b). We conclude that alterations in striatal D2Rs are sufficient to alter movement, but not caloric balance or body weight in mice.

Discussion

Obesity is associated with physical inactivity, which is also believed to contribute to weight gain. Additionally, increased adiposity is hypothesized to contribute to low activity levels in people with obesity (Ekkekakis and Lind, 2006; Westerterp, 1999), although this idea is difficult to test directly. Interestingly, people who lose weight either through diet (de Boer et al., 1986; de Groot et al., 1989; Martin et al., 2007; Redman et al., 2009) or bariatric surgery (Berglind et al., 2015a; Berglind et al., 2015b; Bond et al., 2010; Ramirez-Marrero et al., 2014) do not increase their activity levels, arguing against the weight of adiposity causing their inactivity. Here, we investigated the hypothesis that diet-induced obesity causes physical inactivity via deficits in striatal DA transmission. Consistent with previous work, we found that chronic high-fat diet decreased striatal D2R binding (Hajnal et al., 2008; Huang et al., 2006; Narayanaswami et al., 2013; van de Giessen et al., 2012; van de Giessen et al., 2013). We also observed a deficit in motor-related firing of striatal neurons in obese mice. Inhibiting iMSNs with a G_i-coupled DREADD rescued activity in obese mice, demonstrating that mice with excess adiposity can move normally when basal ganglia output is restored. Surprisingly, however, neither basal D2R measurements nor physical activity correlated with weight gain, a point we observed in multiple experiments. This is in contrast to a study in rats, which may reflect species or experimental differences (Michaelides et al., 2012). We conclude that reductions in D2Rs, and subsequent physical inactivity, are consequences of obesity, but are not necessarily causally linked to further weight gain in mice.

A link between altered D2R signaling and obesity was first identified in humans, and was initially replicated by others (de Weijer et al., 2011; Kessler et al., 2014; Volkow et al., 2008;

Wang et al., 2001). However, more recent work has called this finding into question (Caravaggio et al., 2015; Cosgrove et al., 2015; Dunn et al., 2012; Guo et al., 2014; Karlsson et al., 2015a; Karlsson et al., 2015b; Steele et al., 2010; Tuominen et al., 2015). Although additional research is needed to understand the discrepancies observed among clinical studies, they may reflect complexities inherent to clinical studies and PET imaging. For example, raclopride, the radioligand used in many studies can be displaced by endogenous DA and therefore binding can be influenced by differences in basal DA tone (Horstmann et al., 2015). In addition, the relationship between D2R levels and obesity may be non-linear, such that changes in D2Rs may occur differently in patients with differing levels of obesity (Horstmann et al., 2015). Finally, factors such as sleep duration (Wiers et al., 2016) and caffeine intake (Volkow et al., 2015) can also affect D2R binding, and are not reported or controlled in most clinical studies. These sources of variance can be mitigated in animal studies, which paint a consistent picture of reductions in D2R mRNA (Mathes et al., 2010; Zhang et al., 2015), protein (Adams et al., 2015; Johnson and Kenny, 2010), and receptor binding (Hajnal et al., 2008; Huang et al., 2006; Narayanaswami et al., 2013; van de Giessen et al., 2012; van de Giessen et al., 2013) in obese rodents. Our work extends this body of literature by reporting that other aspects of DA signaling remain unchanged in obese mice, even those with reductions in D2Rs. Additionally, given our observed reduction in D2R binding of ^{3}H-spiperone, but no change in total D2R protein or *Drd2* mRNA, we believe that alterations to the D2R may involve post-translational changes such as receptor internalization. Although our data suggest that reduced D2R binding is sufficient to decrease physical activity in obesity, physical activity is influenced by many factors including genetics and environment (Bauman et al., 2012). We believe it is unlikely that the D2Rs are the only neurological change associated with physical inactivity in obesity. For instance, changes in circulating hormones such as ghrelin, leptin, and insulin act on dopaminergic neurons and may influence activity (Murray et al., 2014). Finally, although we did not observe changes in D1Rs, we cannot rule out changes in neuronal firing of direct pathway neurons that may also influence physical activity.

It is unclear whether variation in D2R availability predisposes individuals to gain weight. Humans with the *Drd2* Taq1A allele have reduced D2R availability and an increased risk of obesity (Blum et al., 1996; Carpenter et al., 2013; Noble et al., 1991; Stice et al., 2008; Thompson et al., 1997). In addition, mice with a global deletion of D2Rs more readily gained weight on a high-fat diet, which was attributed to physical inactivity (Beeler et al., 2015). In contrast, individual variation (natural or genetically induced) in striatal D2R correlated with activity levels in our study, but neither correlated with weight gain. An important distinction in our study was that our genetic model removed D2Rs solely from iMSNs. In addition, careful measurements of food intake and energy expenditure revealed that manipulating D2Rs on these neurons did not alter energy balance. As such, studies that demonstrate links between global D2R function and energy balance may be observing the effects of D2Rs on other cell types. Our experiments support the conclusion that physical inactivity is a consequence of obesity, but in itself is not sufficient to cause changes in weight.

Despite the growing evidence that physical activity is associated with improvements in cardiovascular health and decreased risk for several other chronic diseases, physical activity

remains low in individuals with obesity (Ekkekakis et al., 2016). The lack of effective interventions for increasing physical activity levels is reflected in a lack of understanding of the cellular and molecular mechanisms underlying physical inactivity in individuals with obesity. Here we link physical inactivity to changes in basal ganglia function, providing a biological explanation for the lack of physical activity in individuals with obesity.

Experimental Procedures

Subjects and diets

In all studies, mice were individually housed under standard conditions (12 h light/dark cycle, 21–22°C), with *ad libitum* access to food and water. Mice were provided either standard chow diet (5001 Rodent Diet, 3.00 kcal/g with 29% energy derived from protein, 13% from fat, and 56% from carbohydrate; LabDiet, St. Louis, MO) or high-fat diet (D12492, 5.24 kcal/g with 20% energy derived from protein, 60% from fat, and 20% from carbohydrate; Research Diets, Inc., NJ). All procedures were performed in accordance with guidelines from the Animal Care and Use Committee of the National Institute on Diabetes and Digestive and Kidney Diseases.

Transgenic conditional knockout iMSN-*Drd2*-KO mice were generated by crossing mice expressing Cre driven by regulatory elements of the adenosine 2A receptor gene (*Adora2a*) (B6.FVB(Cg)-Tg(Adora2a-Cre)KG139Gsat/ Mmucd, GENSAT, 036158-UCD) with mice carrying conditional *Drd2* null alleles B6.129S4(FVB)-*Drd2*$^{tm1.1Mrub/J}$,JAX020631 (Bello et al., 2011).

Body composition and energy expenditure calculations

Body composition was measured every other week using ^{1}H NMR spectroscopy (EchoMRI-100H; Echo Medical Systems LTD, Houston, TX). Energy expenditure was determined using an energy balance calculation (Guo et al., 2009; Ravussin et al., 2013):

$$\text{Energy expenditure} = \text{Metabolizable energy intake} - (\Delta \text{ fat mass} + \Delta \text{ fat-free mass}).$$

Open field activity

Open field tests were conducted in PhenoTyper cages (30 × 30 cm; Noldus IT, Leesburg, VA), and EthoVision video analysis software (Version 11; Noldus IT, Leesburg, VA) was used to track mice throughout testing.

Home cage wheel running

Wheel running was measured by placing low profile wireless running wheels (Med Associates, Inc., St. Albans, VT) into the mice's home cages for 72 h every 3 weeks (diet-induced obesity experiments) or continuously (iMSN-*Drd2*-KO experiments).

Blood measures

Ocular vein blood from sacrificed animals was used for the analysis of serum metabolites and hormones after a 4h fast.

Dopamine receptor autoradiography

Right hemisections were cryosectioned at the level of the striata (−0.22, 0.14, 0.62, and 1.18 mm from bregma, covering the full extent of the striatum) into 12 mm sections. Slides were thawed and preincubated in assay buffer (20 mM Hepes, 154 mM NaCl, and 0.1% bovine serum albumin (BSA); pH 7.4) for 20 min at 37°C. D1R binding was assessed by incubating slides in assay buffer containing 1.5nM tritium-labeled SCH-23390 (Perkin-Elmer; Waltham, MA) and 100 nM ketanserin for 60 min at 37°C. D2R binding was assessed by incubating slides with 600pM tritium-labeled spiperone (Perkin-Elmer) and 100nM ketanserin for 100 min at 37°C. Following incubation with the appropriate radioligand, slides were washed twice for 10 min at 4°C in wash buffer (10 mM, Tris-HCl, 154 mM NaCl) then dipped in water (0°C) and allowed to dry overnight. Slides were then exposed to phosphor imaging plates for 7 (D1R binding) or 11 days (D2R binding) and developed using a phosphoimager (Cyclone, Perkin-Elmer, Waltham, Massachusetts). For analysis, areas of interest were outlined and analyzed using Optiquant image analysis software (Perkin-Elmer).

Western blotting

Western blots were incubated with mouse anti-D2DR antibody (1:500, Santa Cruz, sc-5303) or mouse anti-GAPDH antibody (1:1000, Santa Cruz, sc-32233) and after that with goat antimouse IgG-HRP (1:1000, Santa Cruz, sc-2005). Chemiluminescence signal was generated using enhanced chemiluminescence western blotting detection reagents (Bio-Rad) and visualized with Chemidoc Imaging System (Bio-Rad).

In situ hybridization

An RNAscope multiplex fluorescent assay kit was used for *in situ* hybridization (Advanced Cell Diagnostics, Newark, CA). Briefly, formalin-fixed sections were dehydrated in ethanol followed by protease exposure. Sections were then hybridized with RNAscope oligonucleotide probes against *Drd2*. Following probe hybridization, slides were incubated with signal amplifier according to RNAscope protocols. Slides were then washed with RNAscope wash buffer. Finally, slides were mounted with DAPI counterstain.

High-performance liquid chromatography with electrochemical detection

Left hemisections were processed for detection of DA using reverse-phase HPLC-EC, as previously described (Kilpatrick et al., 1986).

Tyrosine hydroxylase immunohistochemistry

Slide mounted sections were fixed in 10% neutral buffered formalin, rinsed in 0.1 M TBS pH 7.5 and incubated in a primary antibody solution containing 3% normal donkey serum, 0.3% Triton-100, and rabbit anti-tyrosine hydroxylase antibody (1:1000, Millipore #MAB152) overnight at 23°C. The following day, tissue sections were rinse d in TBS, and incubated in a secondary antibody solution containing 3% normal donkey serum, 0.3% Triton X-100, and goat anti-rabbit conjugated to Alexa-Fluor 555 (Millipore AQ132F). For each mouse, two striatal sections were analyzed, except for four mice (two HFD, two Chow) where only one section was analyzed due to poor tissue or image quality.

MicroPET

Mice were injected with ^{18}F-fallypride with a specific activity of 2.5 ± 0.34 mCi/nmol in a volume of 130μl via tail vein while under isoflurane anesthesia. The microPET scan was carried out for 2 hours, during which 25 frames were acquired for analysis. The time-activity curves for ^{18}F-fallypride in the ROIs were extracted using AFNI software (http://afni.nimh.nih.gov/afni) and kinetic parameters were fit to a four-compartment model using a custom Matlab script (with the cerebellum used as the reference tissue) to determine the D2R binding potential (Lammertsma and Hume, 1996).

In vivo electrophysiology

Recordings were made from an electrode array containing 32 Teflon-coated tungsten microwires (35 mm diameter) implanted unilaterally in the dorsomedial striatum (A/P: +0.8; M/L: +1.5; D/V: −2.6 mm per bregma), and processed with commercial software (Offline Sorter and Neuroexplorer, Plexon, Inc).

Stereotaxic viral vector injection

Mice were briefly anesthetized via isoflurane exposure. Once deeply anesthetized, a single incision was made along the midline, the skull was exposed, and a bilateral craniotomy was made (A/P: +0.5, M/L: +/− 1.5 mm per bregma). Viral vector containing the inhibitory KOR-DREADD (Syn-DIO-hKORD-IRES-mCit-WPRE; 0.5 μl) was injected bilaterally into dorsomedial striatum (DV −2.8 mm from the top of the skull) and allowed to express for 9 weeks prior to experimentation.

No-net flux microdialysis and dopamine analysis

Measurements of basal extracellular DA, DOPAC and homovanillic acid (HVA) in the dorsal striatum of mice were performed by no-net flux microdialysis approach. Unilateral 2 mm probes (18 kDa membrane cut-off) were stereotaxically implanted 1 week after cannula implantation with continuous perfusion of artificial cerebrospinal fluid (aCSF) at 1 μl/min for 4h before sample collection (see Supplementary Experimental Procedures). No-net flux experiment to measure extracellular DA levels was performed by randomly perfusing six different concentrations of DA (0, 2.5, 5, 10, 20, and 40 nM) in aCSF through the dialysis probe. Each DA concentration was perfused for 30 min, and then 2×10 min samples collected in 2.5 μl 100mM HCl + 1mM EDTA to prevent catecholamine degradation and frozen at −80°C. For neurochemical analyses, isocratic HPLC system coupled to amperometric detection was used (HPLC-EC; BASi LC-4C, West Lafayette, IN). Only mice with proper probe placement were included in the analysis (Figure S3e).

Statistics

Statistical analysis was performed using GraphPad Prism (Version 6.07; GraphPad Software Inc., La Jolla, CA). Unless stated, two-tailed student's *t*-tests were used. Otherwise, two-tailed paired *t*-tests, one-way repeated-measures ANOVAs or two-way repeated measures ANOVAs were used when appropriate and as stated. ANOVAs were followed by t-tests for post-hoc comparisons. Results were considered significant at an alpha of $p < 0.05$, or with alpha determined by Bejamini-Hochberd FDR correction, where appropriate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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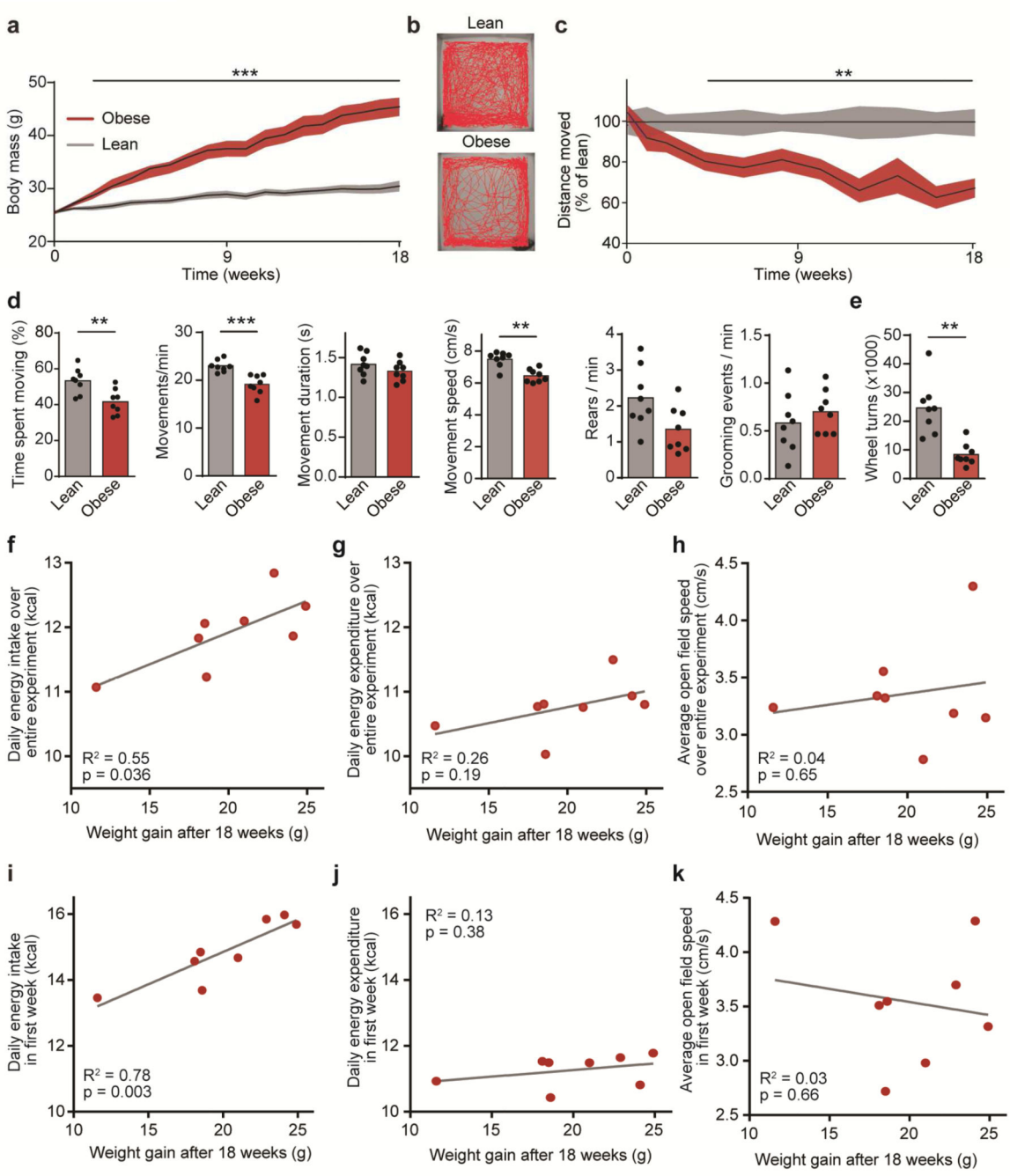

Figure 1. Chronic high-fat diet led to physical inactivity

(**a**) Mice fed a high-fat diet weighed more than mice fed standard chow beginning at week 2 and continuing to week 18 ($F_{(18, 252)} = 62.43$, $p < 0.0001$). (**b, c**) Obese mice have reduced physical activity compared to lean mice beginning at week 4 and continuing until week 18 ($F_{(10, 140)} = 4.83$, $p < 0.0001$). (**d**) After 18 weeks on high-fat diet, obese mice had decreased time spent moving ($t_{(14)} = 3.32$, $p = 0.005$), decreased frequency of movement ($t_{(14)} = 4.74$, $p = 0.0003$), and speed while moving ($t_{(14)} = 4.69$, $p = 0.0002$) relative to lean controls. Obese mice also showed a trend for decreased rearing ($p = 0.07$). (**e**) When given access to a

running wheel in the home cage, obese mice had fewer wheel turns relative to lean mice ($t_{(14)} = 4.55$, $p = 0.0005$). (**f–h**) Energy intake over the course of the experiment ($r = 0.74$, $p = 0.04$), but not energy expenditure (r = 0.52, p = 0.19) nor open field speed (r = 0.19, p = 0.65), formed a significant correlation with total weight gain. (**i–k**) Average energy intake during the first week ($r = 0.88$, $p = 0.004$), but not energy expenditure ($r = -0.19$, $p = 0.66$), nor open field speed ($r = 0.36$, $p = 0.38$), formed a significant correlation with total weight gain. (**a, c**) two-way repeated measures ANOVA followed by posthoc t-test with Benjamini Hochberg false discovery rate; (**d–e**) unpaired student's *t*-test; (**f–h**) linear regression; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$ vs. lean. (**i–k**) linear regression; ***$p < 0.001$ vs. lean mice.

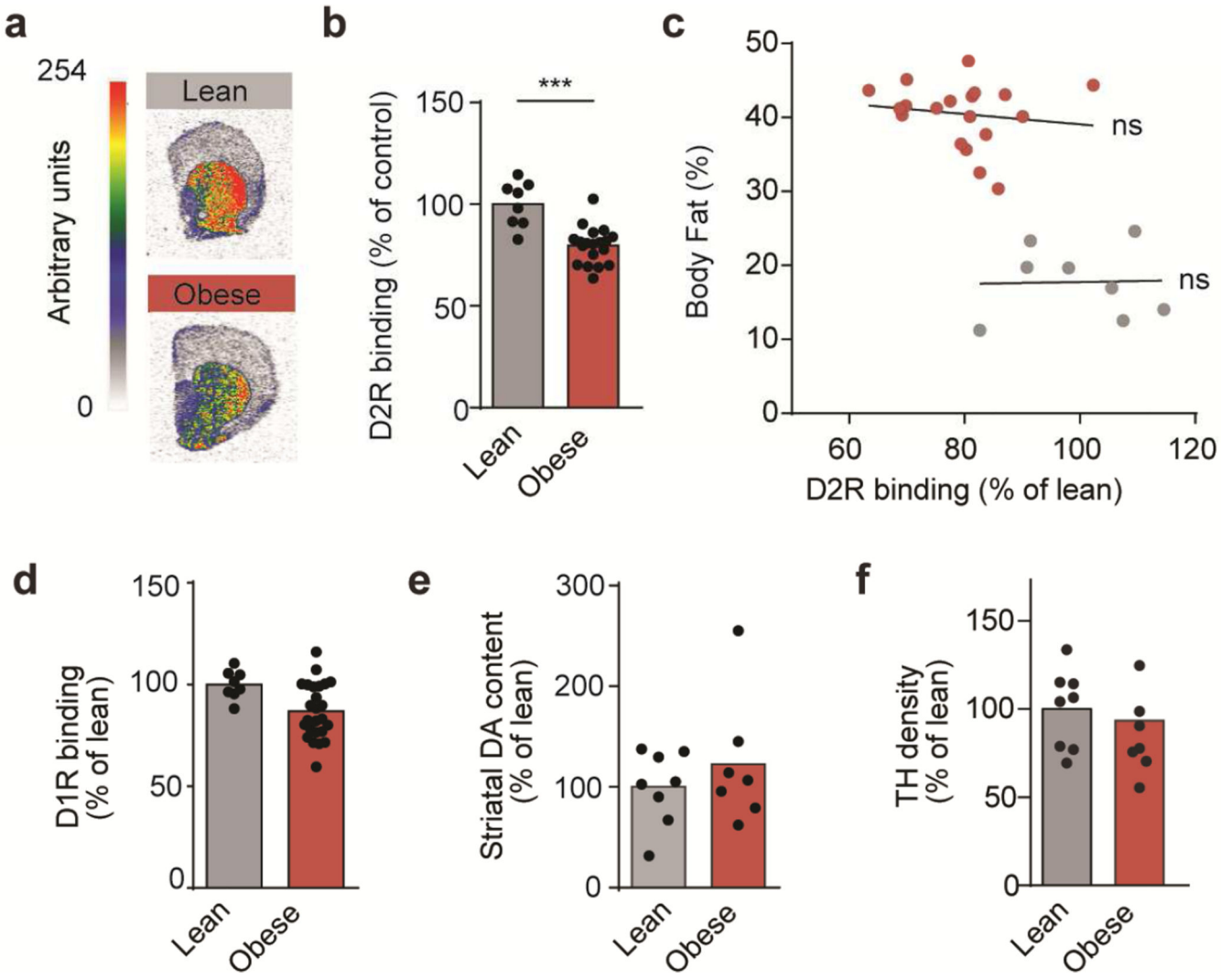

Figure 2. High-fat diet impaired striatal dopamine D2R binding

(**a**) Images of striatal D2R binding as measured via ^{3}H-spiperone autoradiography. (**b**) Striatal D2R binding was decreased in obese relative to lean mice ($t_{(25)} = 5.02$, $p < 0.0001$). (**c**) Striatal D2R binding was not correlated with body fat percentage in lean ($p = 0.95$) or obese mice ($p = 0.56$). (**d–f**) Striatal D1R binding ($t_{(24)} = 1.31$, $p = 0.20$), total dopamine content (DA; $t_{(13)} = 0.85$, $p = 0.41$), and tyrosine hydroxylase (TH) density ($t_{(14)} = 0.48$, $p = 0.64$) were not different between diet groups. Mean with individual mice; $n = 8–19$ mice/group; student's t-test (**b, d–f**) or linear regression (**c**); *$p < 0.01$.

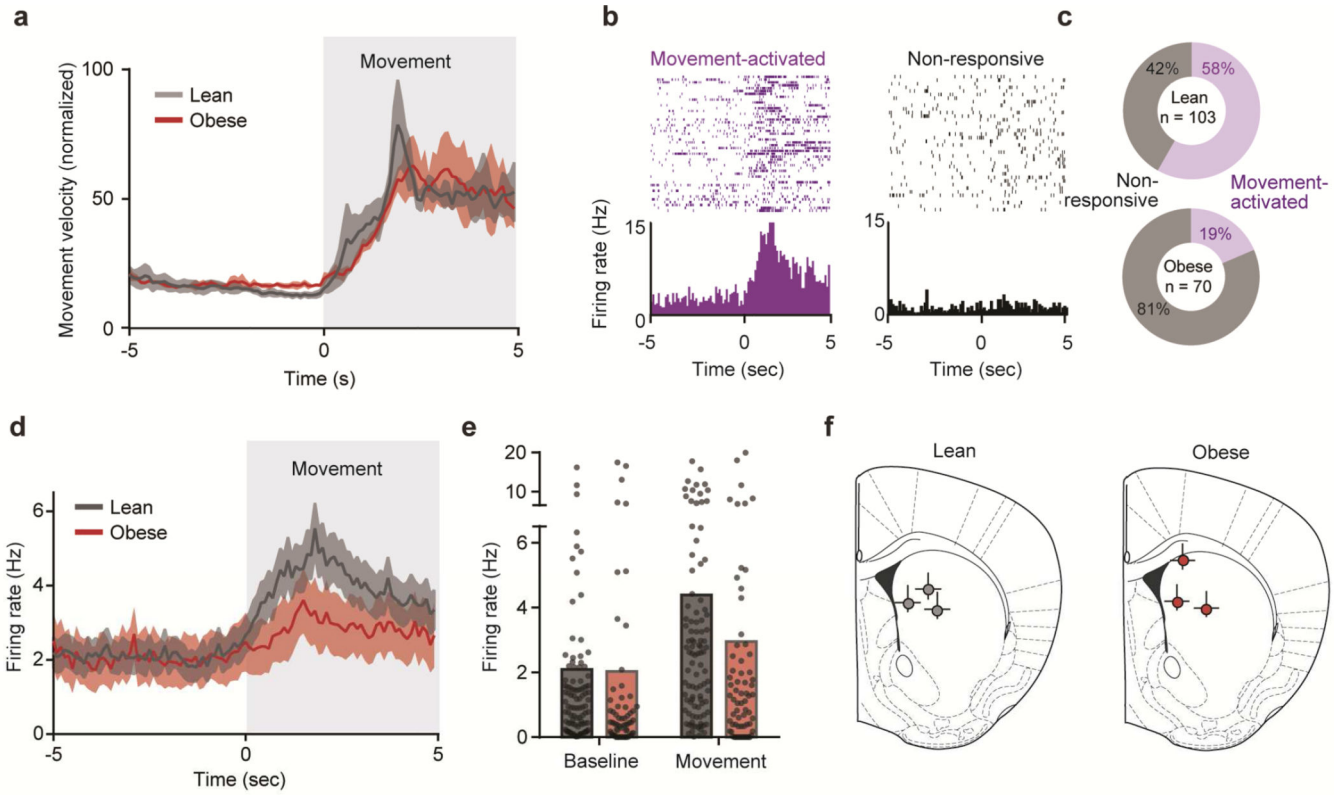

Figure 3. Movement-related firing in the striatum was disrupted in obese mice

(**a**) Movement events had similar velocity in lean and obese mice (**b**) Examples of movement-activated and non-responsive firing in striatal neurons. (**c**) Prevalence of movement-activated neurons was lower in obese mice ($p = 0.002$). (**d, e**) Movement-related firing of all recorded neurons was significantly lower following diet exposure (diet × movement interaction, $F_{(1, 171)} = 14.77$, $p < 0.0002$). (**f**) Schematic (adapted from (Franklin and Paxinos, 1997)) illustrating electrode array placement in lean and obese recording mice (n = 3 each). (**c**) Fisher's exact test (**d–e**) two-way repeated measures ANOVA.

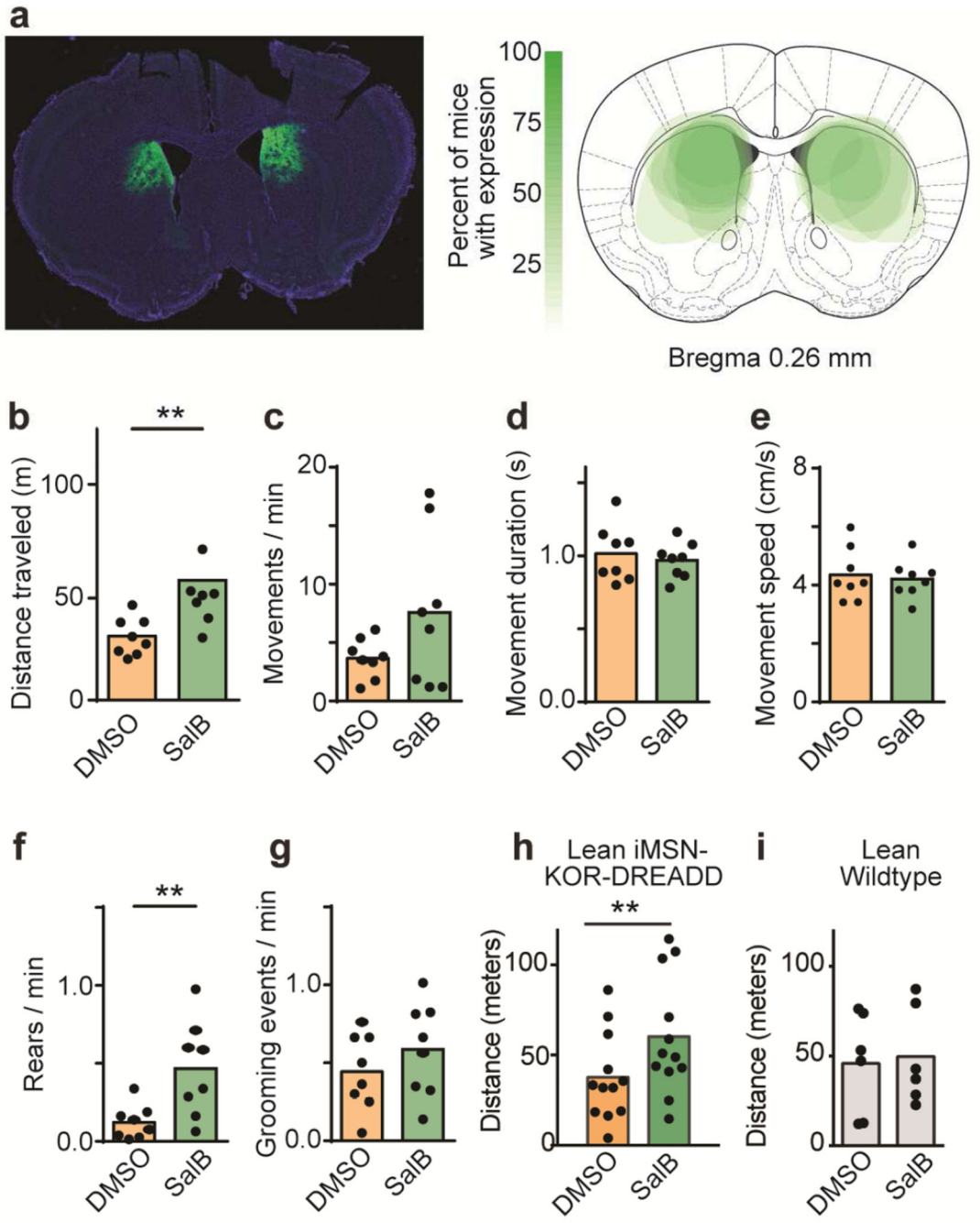

Figure 4. DREADD mediated inhibition of iMSNs restored physical activity in obese mice

(**a**) Photograph of KOR-DREADD expression, and schematic (adapted from (Franklin and Paxinos, 1997)) illustrating viral injection sites of all KOR-DREADD in A2A-Cre mice; opacity indicates number of mice expressing virus in a given location. (**b**) Obese mice moved more when injected with SalB compared to DMSO ($t_{(7)}$ =3.056, p = 0.02). (**c–g**) After SalB administration, obese mice increased their frequency of rearing ($t_{(7)}$ = 3.116, p = 0.02), and trended towards an increase in frequency of movements ($t_{(7)}$ =1.64, p = 0.12) relative to when administered DMSO. (**h**) Lean mice moved more when injected with SalB

compared to DMSO ($t_{(9)}$ =3.3, p = 0.01). (**i**) SalB did not affect movement in wildtype mice that did not express the KOR-DREADD (p=0.77). (**b–i**) Paired student's t-tests; mean with individual mice; n = 6–10 mice/group.

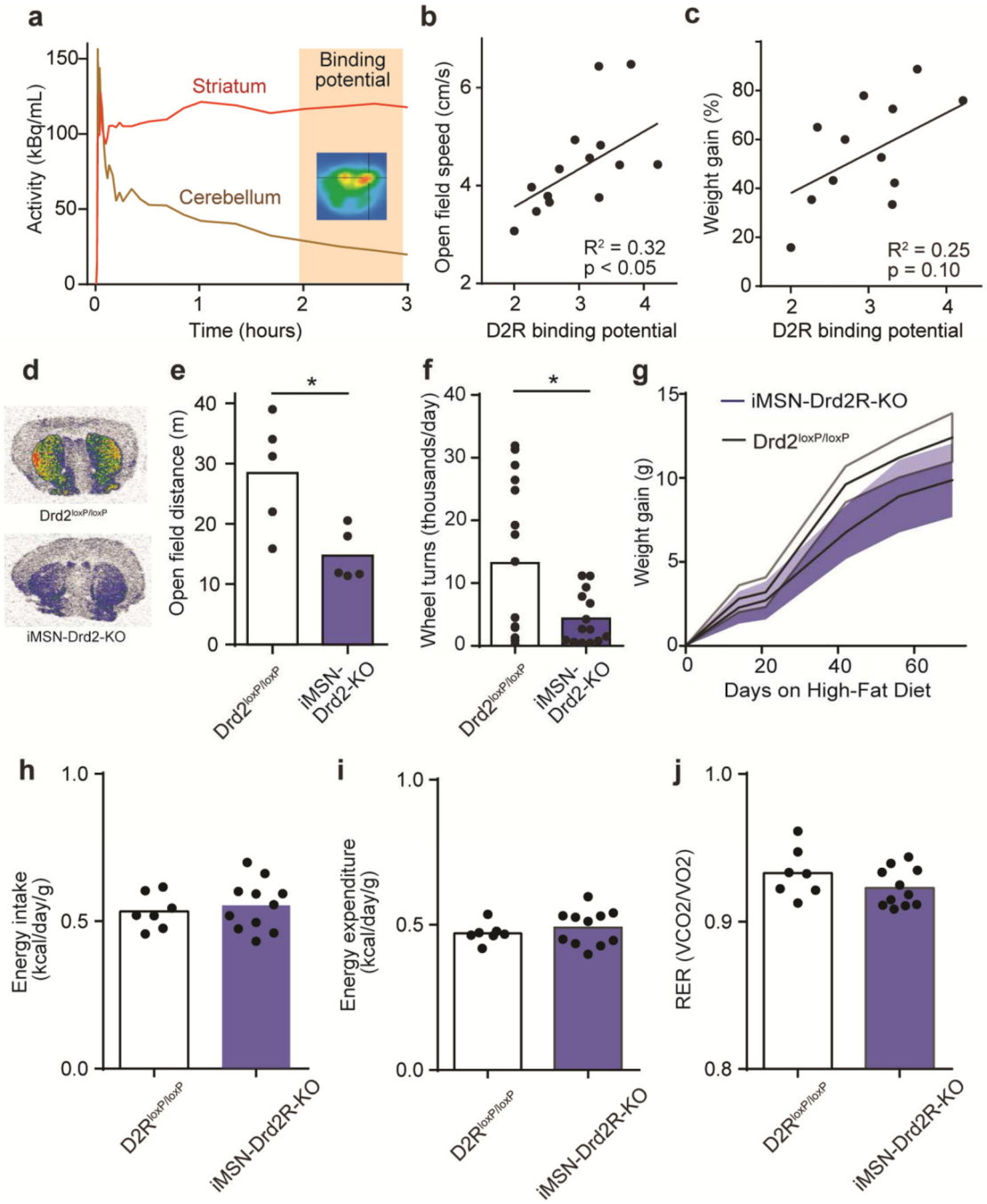

Figure 5. Basal D2R binding did not predict future weight gain

(**a**) Example D2R microPET availability curves in the striatum and cerebellum using ^{18}F-fallypride. (**b–c**) D2R binding potential correlated with basal open field movement ($r = 0.56$, $p = 0.045$), and trended towards a positive relationship with high-fat diet-induced weight gain ($r = 0.50$, $p = 0.10$, $n = 12–14$ mice). (**d**) Representative D2R autoradiography in mice with intact D2Rs (top) and iMSN-*Drd2*-KO mice (bottom). (**e–f**) iMSN-*Drd2*-KO mice had decreased physical activity in an open field ($t_{(8)} = 2.99$, $p = 0.02$) and on home-cage running wheels ($p = 0.01$, $n = 5–19$ mice/group). (**g**) iMSN-*Drd2*-KO mice and *Drd2*-floxed

littermate controls gained similar amounts of weight on high-fat diet ($F_{(5, 70)} = 1.417$, $p = 0.23$; $n = 6–10$ mice/group). (**h–j**) There were no difference in normalized energy intake ($p = 0.60$), energy expenditure ($p = 0.47$), or RER (p = 0.17) between iMSN-D2R-KO mice and littermate controls.(**b–c**) linear regression; (**e–f, h–j**) unpaired Student's t-test, (**g**) two-way repeated measures ANOVA, $*p < 0.05$.