Metaplasticity in the ventral pallidum as a potential marker for the development of diet-induced obesity

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Abstract

Background: A major driver of obesity is the increasing palatability of processed foods. Although the reward system promotes the consumption of palatable food it is not yet understood whether and how pathology in the reward system may drive obesity. Here, we examine how chronic high-fat-high-sugar (HFHS) diet changes the physiology of the ventral pallidum (VP), a key hub in the reward system, how obesity-prone mice differ from obesity-resistant mice in their VP physiology, and identify metaplasticity in the VP as a possible marker for the predisposition to overeat.

Methods: Male C57bl6/J wildtype mice first had 10-12 weeks of chronic exposure to HFHS or chow diet in their homecages. Then, mice were sacrificed and we recorded from single VP neurons in acute slices using the whole-cell patch clamp technique.

Results: Chronic HFHS diet decreased the excitability of VP neurons, but without major change in synaptic inhibitory input. Within the HFHS group, obesity-prone (OP, top 33% weight gainers) mice had a less excitable VP compared to obesity-resistant (OR, bottom 33% weight gainers) mice and showed short and long term potentiation of incoming inhibitory inputs. Moreover, we found that long term potentiation of the inhibitory inputs to the VP may exist in OP mice before exposure to HFHS and may thus serve as a marker for the predisposition to become obese.

Conclusions: Our data points to the VP as a critical player in obesity and suggest that hypoactivity in the VP may play a significant role in promoting overeating of palatable food.

Introduction

As society moves forward, western, calorie-rich, diet is becoming more and more common in households. Consequently, obesity has become a world epidemic and a leading cause of preventable death worldwide (1) . Despite the drastic increase in obesity levels, the biological mechanisms driving this epidemic are still not understood and research is being conducted in many disciplines, including psychology, metabolism and neurobiology.

One main theory about the burst of the obesity epidemic in recent decades is that food has become during these years an increasingly strong reinforcer (2). Various studies have indeed shown that not only do palatable foods act as reinforcers and can activate the reward system

(3), but that direct activation of specific neurons in the reward system can drive by itself motivated feeding behavior (4–6). Such a strong link between the reward system and consumption of palatable foods poses the possibility that overeating of palatable food, leading to obesity, may involve pathologies in the reward system akin to those seen in models of addiction.

Research so far has indeed shown obesity-related changes both in the function of the reward system and in motivation-driven food seeking behavior. Thus, the expression level of the dopamine receptor D2/3 in the striatum is decreased in obese humans (7–9) and rodents (6, 10); opioid signaling in both the ventral tegmental area (VTA) (11, 12), and the nucleus accumbens (NAc) (12, 13) is altered in obese rodents; and the glutamatergic input to the NAc is potentiated in rats that develop diet-induced obesity (14, 15), akin to the effect seen after withdrawal from cocaine (16, 17) or nicotine (16, 18). Possibly as a consequence of these changes in the reward system, rats and mice that gain the most weight in a diet-induced obesity model also show the highest motivation to obtain palatable food (10, 14, 19–21).

A key structure in the reward system which is yet to be studied in the context of obesity is the ventral pallidum (VP). The VP is a key junction between the reward system and the main hub for feeding behavior, the lateral hypothalamus (LH) (22, 23). It receives strong inhibitory input from the NAc (24, 25) and in turn sends inhibitory projections to the LH and other targets (26). Thus, it is not surprising that the VP has an important role in modulating feeding behavior (23, 27–29). Nevertheless, whether it has a role in pathological overeating and obesity has not been studied.

In this study we set to examine whether chronic consumption of HFHS diet alters the physiology of the VP and whether mice that are more prone to gain weight when HFHS food is freely available exhibit different VP physiology than those that are more resistant to gaining weight. Moreover, using behavioral and electrophysiological tools we test whether the differences in VP physiology between obesity-prone and resistant mice may be innate and thus possibly be part of the mechanism promoting overeating and weight gain.

Results

High Fat High Sugar diet diminishes excitability of VP neurons

In order to establish the long-lasting influence of chronic consumption of highly palatable food on the VP of mice, we exposed two groups of mice to different diet protocols. The first group received high-fat-high-sugar (HFHS) diet *ad libitum* in the home cage for 10-12 weeks followed by two weeks of standard chow diet. The second group received standard chow for a similar time period (Figure 1A).

We then examined, using whole-cell patch clamp electrophysiology, whether the long exposure to HFHS had affected the excitability of VP neurons (Figure 1B). We found that both resting membrane potential (Figure 1C) and action potential firing frequency (Figure 1D-E) were significantly lower in the VP of HFHS mice compared to the Chow mice. Moreover, we found that when depolarized to different levels, VP neurons of HFHS mice fired significantly less than VP neurons of Chow mice, an effect that was most robust at the strongest depolarization steps (+60 and +80 mV) (Figure 1F,H). The minimal latency to the first action potential in a depolarizing step, an additional measure of excitability, was not different between groups (Figure 1G). These data demonstrate that VP neurons of mice that fed on HFHS diet for 10-12 weeks showed a marked decrease in their excitability measures, that was present two weeks after the last exposure to HFHS.

High fat high sugar diet impairs GABA neurotransmission in the VP

The changes in cellular excitability induced by the chronic HFHS diet may be accompanied by changes in synaptic neurotransmission in the VP. To test for that we compared evoked and spontaneous inhibitory postsynaptic currents (eIPSCs and sIPSCs, respectively) between the HFHS and the Chow groups. When examining the amplitude and frequency of sIPSCs, we found that chronic HFHS diet significantly decreased the amplitude (Figure 2A,C) but not the frequency (Figure 2B,C) of sIPSCs. As changes in sIPSC amplitude are indicative of postsynaptic mechanisms, these data suggest that chronic HFHS diet decreases the postsynaptic sensitivity of VP neurons to GABA neurotransmission.

To examine whether chronic HFHS diet changed also evoked GABA neurotransmission in the VP we examined the generation of short and long term synaptic plasticity. Short term plasticity was induced by applying 5 identical pulses at 1 Hz, 10 Hz, or 50 Hz and the change in eIPSC during the protocol was calculated by dividing each eIPSC by the first one. Our data show that the HFHS diet did not affect short-term plasticity in the VP in either of the frequencies (Figure 2D,E; data for 1 Hz not shown). To induce long term plasticity, we applied a high frequency stimulation (HFS) protocol that we used before (30) to elicit LTD in the VP. We found that the long-term effect of the HFS protocol on the eIPSC amplitude did not differ between HFHS and Chow mice (Figure. 2F-H). Overall, the data suggest that chronic HFHS diet diminishes spontaneous neurotransmission through a postsynaptic mechanism but does not affect the short- and long-term plasticity of evoked GABA neurotransmission.

Obesity-prone mice show decreased excitability in the VP compared to obesity-resistant mice

Our data so far show that chronic exposure to HFHS food changes the physiology of the VP. Our next question was to determine whether within the group of mice exposed to HFHS diet, the physiology of the VP differed between those mice that gained the most weight (top 33% weight-gainers relative to day 1 of the HFHS diet, called obesity-prone, OP) and those who gained the least weight (bottom 33% weight gainers, called obesity-resistant, OR) (Figure 3A). Previous studies showed that not only does weight gain correlate with addiction-like behaviors towards palatable food (14, 15, 20), it also correlates with synaptic changes in the NAc (14, 15) - OP rats show stronger excitatory input to the NAc compared to OR rats. As the VP is a major target of the NAc, we expect that the physiology of the VP will also differ between OP and OR mice.

Membrane potential of VP neurons was significantly more hyperpolarized in OP mice compared with OR mice (Figure 3B). Action potential frequency was not significantly different between OP and OR mice, although note that the average firing frequency was lower in VP neurons of OP mice by more than 20% (mean ± SD, 5.20 ± 4.9 and 4.07 ± 7.4 for OR and OP mice, respectively) (Figure 3C-D). Applying depolarizing steps of increasing amplitudes from baseline membrane potential induced a comparable number of action potentials in both groups (Figure 3E), but the

minimal latency to the first action potential, a measure inversely correlated with cellular excitability (31), was significantly longer in the OP mice (Figure 3F-G). Overall, these data show that VP neurons of OP mice may have decreased excitability compared with those of OR mice.

Obesity-prone mice show altered synaptic plasticity in GABA input to the VP

To evaluate whether GABA synaptic transmission in the VP is also different between OP and OR mice we compared spontaneous and evoked IPSCs with the same tools as in Figure 2. An examination of the amplitude and frequency of the inhibitory spontaneous events in the VP showed that these parameters were comparable between OP and OR mice (Figure 4A-C). The ability of VP neurons to show short-term plasticity, on the other hand, was different between OP and OR mice. Application of 5 consecutive stimulations at 10 or 50 Hz (but not at 1 Hz, not shown) induced a strong potentiation of the IPSC in the OP mice but not in OR mice (Figure 4D-E). This could mean that in OP mice, but not OR mice, fast repeated activation of inhibitory inputs to the VP may result in stronger inhibition that builds up with activity.

VP neurons of OP and OR mice also showed a striking difference in their long-term response to the high-frequency stimulation (HFS). Application of the HFS protocol in VP neurons of OR mice decreased the average IPSC amplitude to 67.4±45% of baseline, but this did not reach significance (5 out of 6 cells showed a transient depression but one cell showed potentiation) (Figure 4F-H)(30). In contrast, the HFS protocol induced a long-term potentiation (LTP) in VP cells of OP mice (Figure 4F-H). Note the similarity in the results for the long and the short-term plasticity protocols - in both cases the inhibitory input to the VP of OP mice was potentiated while the input to the VP of OR mice did not change or even showed depression in some cells. Overall, the data in Figure 4 show a difference in short- and long-term plasticity of inhibitory input to the VP between OP and OR mice, with a tendency towards potentiation of these inputs in OP mice.

Differences in long-term plasticity between OP and OR mice may be innate

In this and previous works, we and others showed various synaptic and physiological parameters in the NAc and the VP that differ between OP and OR animals (14, 15). For obvious reasons, slice recordings in all these studies could only be performed *after* the HFHS diet and splitting mice to OP and OR groups. This technical hurdle left one of the most important questions unanswered - are these synaptic differences between OP and OR mice the cause or the consequence of obesity?

In a recent study (20) we showed that in a progressive ratio task where the reward is a small pellet of HFHS food, head entries to the food receptacle may serve as a good predictor for being prone to develop obesity (20). Accordingly, mice that eventually were defined as OP mice showed significantly more head entries than OR mice even when the test was conducted before the beginning of the HFHS diet. Based on this, we hypothesize here that if a physiological difference in the VP between OP and OR mice is innate, it may also be observed when comparing HFHS-naive mice that show the highest and the lowest rates of head entries in a progressive ratio task.

When choosing the physiological parameters to test, we specifically looked for parameters that on the one hand were not affected by the mere exposure to HFHS diet but on the other hand did differ between OP and OR mice after prolonged HFHS consumption. This is because we looked for innate differences between OP and OR mice. Thus, any difference driven by the HFHS diet itself (Figure 1-2) cannot be confirmed as being innate. Based on these a-priori conditions, we examined the only physiological measures that fulfilled the requirements - the minimal latency to the first action potential when depolarizing the cells and the long and short - term plasticity.

To examine whether the difference between OP and OR mice in either of the three physiological measures listed above is innate, we trained a chow group in a progressive ratio task. Briefly, mice were first trained to press a lever in order to receive a 20 mg pellet of chow food (see Methods for details) and then were left in their home cages for 10-12 weeks with regular chow diet (Figure 5A). Then, their performance (head entries into the food receptacle and active lever presses) was measured during a progressive ratio task - the number of lever presses required for the delivery of a 20 mg pellet increased progressively until mice did not

reach the next requirement. We then split the mice to those who showed the highest (top 33%, “high seekers”) and lowest (bottom 33%, “low seekers”) number of head entries (Figure 5B). Note that there was no correlation between the head entries and the lever presses (Figure 5C), confirming our previous finding that prior to the chronic consumption of HFHS diet head entries may be a better predictor of future weight gain than operant food seeking (20).

The minimal latency to the first action potential in a depolarizing step and the short term plasticity of evoked GABA release showed no difference between high- and low-seekers (Figure 5D-E), unlike the results in OP and OR mice (Figures 3-4). This suggests that these differences in excitability and short term plasticity of VP neurons may be a consequence of the HFHS diet rather than an innate difference. In contrast, application of the HFS protocol to induce long term plasticity showed a significant difference between high-seekers and low-seekers - it generated LTD in the VP of low-seekers but LTP in high-seekers (Figure 5F-G). This is strikingly similar to the difference in long-term plasticity between OP and OR mice (Figure 4F-H). Indeed, comparing the magnitude of change in IPSC amplitude showed there was no difference between high-seekers and OP mice and between low-seekers and OR mice (Figure 5G). Thus, the nature of long-term plasticity in inhibitory inputs to the VP may be an innate marker that could predict the level of weight gain in a chronic highly-palatable diet, and possibly be part of the cause for the extreme weight gain in OP mice.

Discussion

In this work we showed a tight connection between the physiology of the VP and diet-induced obesity. First, we showed that chronic HFHS diet caused changes in neurons of the VP both in their excitability and in their inhibitory synaptic input. Accordingly, VP neurons in mice fed chronically with HFHS diet showed lower excitability (Figure 1) and impaired GABAergic input (Figure 2). We also showed that the physiology of VP neurons differs between obesity prone and resistant mice. VP neurons of OP mice were less excitable than those of OR mice (Figure 3) and their inhibitory input tended to potentiate after protocols that induce short and long term plasticity (Figure 4). Lastly, we were able to identify the tendency to show long term potentiation in the VP as a possible innate marker for excessive gain of weight when chronically

exposed to HFHS diet. In a previous study we showed that OP mice seek for palatable food more intensely (higher head entries to the food receptacle) than OR mice even before being exposed to chronic HFHS diet (20). Thus, we used here the intensity of food seeking in HFHS-naive mice as a possible marker for future weight gain. By splitting chow mice by their intensity of food seeking we found that "high seekers" showed LTP akin to OP mice while "low seekers" showed depression comparable in amplitude to that seen in OR mice (Figure 5). Overall our data pose the VP as a central player in diet-induced obesity who may contribute to the differences in diet-induced weight gain between individuals.

The ventral pallidum and obesity

To date there are no studies linking diet-induced obesity to physiological changes in the VP and only few studies have shown changes in the striatum in obese individuals. For example, the expression of the inhibitory D2 dopamine receptor is decreased both in obese humans (9) and animal models of obesity (6, 10). Also, the excitatory input to the NAc is stronger in obese rats (14, 15). Both these findings point to a possible increase in the activity of NAc GABAergic VP-projecting neurons in obesity. This may imply stronger inhibition on the VP in obesity. Indeed, our findings support this hypothesis - VP neurons of OP mice were less excitable than those of OR mice, and their inhibitory input showed a tendency to strengthen unlike that of OR mice. The activity of the VP is crucial in determining food consumption. Blocking VP activity either by lesions or by local injection of GABA agonists decreases food consumption (23, 28, 29), while injecting GABA antagonists into the VP promotes food consumption (28). Likewise, optogenetic activation of VP GABA cells in well-fed mice induces food consumption, and this is true for both chow and high-fat diets (32). The VP was also suggested to encode the hedonic value of food, and indeed blocking GABA neurotransmission increases food and sucrose seeking, but not water or quinine consumption (23, 28, 33) and devaluation of palatable food leads to decreased VP activity upon exposure to it (34).

In spite of all the data linking the VP to feeding, little is known about the role of the VP in obesity. Human studies show an inverse correlation between VP activity to unappetitive images and Body Mass Index (BMI) (35) and higher levels of the serotonin receptor 5HT4R in the VP

and NAc in obese individuals (36). Other studies show a decrease in pallidal grey matter and network efficiency in obese patients (37, 38), although in these studies the globus pallidus is included in the analyses. At this point it is hard to integrate our data, taken at the synaptic level, with present human studies to form a solid hypothesis on the role of the VP in obesity and further research is needed.

Long term plasticity and the susceptibility to develop obesity or addiction

One of our main findings is that VP neurons of OP and OR mice show different long-term plasticity patterns upon application of a HFS (Figure 4). We also show that this difference in long term plasticity patterns may be innate and not caused by the gain of weight (Figure 5). This finding can be paralleled to the changes in metaplasticity (the plasticity of synaptic plasticity (39) sometimes seen in animal models of addiction. The glutamatergic input to the NAc is a plastic input that can show both LTP and LTD in drug naive animals (40). Chronic exposure to cocaine, with or without subsequent withdrawal, impairs this plasticity - both LTD (41–44) and LTP (44) induced by experimental protocols *ex vivo* and *in vivo* in the NAc are abolished. Similarly, we have shown loss of plasticity in the VP after withdrawal from cocaine (30). The impaired plasticity in the NAc seems to not only be a general phenomenon in addiction but to inversely correlate with the susceptibility to develop addiction (43). Thus, rats that show the highest level of cocaine self-administration do not show LTD while rats with minimal cocaine seeking behavior have intact LTD expression. This observation was replicated also in obesity (14). So far it is not known whether the link between plasticity and the susceptibility to develop addiction or obesity is innate or rather a consequence of the chronic consumption of the rewarding agent. Our data points for the first time to the possibility that the differences in long term plasticity in the VP may be an innate marker for the susceptibility to develop obesity.

It remains to be discovered how a tendency to show LTP in inhibitory synapses in the VP may make one more susceptible to overeat than others. A possible hypothesis is that long term plasticity serves as a compensation mechanism against changes in cellular activity. Thus, weakening of the GABAergic input to the VP may compensate for the HFHS-induced decrease in

excitability in the VP. Such weakening may be indeed possible in “low seekers” or some cells of OR mice (Figure 4,5), but OP mice and “high seekers” lack this ability to weaken the inhibitory input to the VP (Figure 4,5) and to correct for the decreased excitability. This may potentially make these latter mice more prone to develop obesity, in a yet unknown mechanism.

Methods

Experimental subjects

Experimental subjects were naive C57bl6/J wildtype male mice weighing between 23g and 30g at the beginning of the experiment. A 12-hour reversed light/dark cycle was maintained, with the lights turned off at 8:00 am. Experimental procedures were conducted during dark hours. Mice were housed individually and nesting/enrichment material was available. Mice were given 7 days to acclimate before experimentation began. All experimental procedures were approved by the Authority for Biological and Biomedical Models in the Hebrew university.

Diet-induced obesity model

After the acclimation period, all mice were put on a 4-week standard chow diet (Teklad Global 2018, 18% kcal fat; total density = 3.1 kcal g^{-1}; Harlan Laboratories Inc., Indianapolis, Indiana), during which body weight and caloric intake were monitored and behavioral training was completed (see below). Then, mice that were put on the HFHS groups (n=24) were switched to a HFHS diet (D12451, 45% kcal fat; total density = 4.73 kcal g^{-1}; Research Diets Inc.) for 10-12 weeks, while the control chow mice (n=15) continued to feed on the standard chow diet. At the end of these 10-12 weeks HFHS mice were switched back to standard chow for 2 additional weeks before being sacrificed for recordings. Body weight was determined twice per week (BJ-410C scales, Precisa, Dietikon, Switzerland) throughout the entire experiment. At the end of the diet-induced obesity protocol HFHS mice were divided according to their weight gain (relative to their weight on Day 1, when they were first exposed to the HFHS diet) into diet-induced obesity-prone (OP, top third weight gainers) and diet-induced obesity-resistant (OR, bottom third weight gainers) groups.

Operant food self-administration protocol

Food self-administration was conducted in mouse operant boxes (MedAssociates, Fairfax VT, USA) as described before (20). Briefly, mice were trained to press a lever in order to obtain a 20 mg precision pellet of chow food (F0071, 5.6% kcal from fat, total density = 3.6 kcal/g; Bioserv Inc., Frenchtown, NJ)) first on a fixed ratio 1 (FR1) schedule (each lever press resulted in a food pellet), and then on FR3 and FR5. Mice were eventually tested at the end of the HFHS diet on a progressive ratio task (where the number of lever presses required to obtain a pellet increased progressively). Progressive ratio schedule was 5, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492 and 603 lever presses per pellet. The test was terminated after 6 hours or if the mouse did not progress to the next level within one hour of achieving the previous one. During the test we monitored the number of lever presses on the active and inactive levers and the number of head entries into the food receptacle (detected by infrared beam). Operant chambers were located in sound-attenuating boxes to minimize external noises. Data was analyzed using Graphpad Prizm 8.2 (GraphPad Software Inc., San Diego, California).

Slice preparation

As described before (45, 46). Mice were anesthetized with 150 mg/kg Ketamine HCl and then decapitated. Sagittal slices (200 µm) of the VP were prepared (VT1200S Leica vibratome) and moved to vials containing artificial CSF (aCSF-in mM: 126 NaCl, 1.4 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 2.5 KCl, 2.0 Na Pyruvate, 0.4 ascorbic acid, bubbled with 95% O_2 and 5% CO_2) and a mixture of 5 mM kynurenic acid and 100 mM MK-801. Slices were stored in room temperature (22-24 °C) until recording.

In vitro whole cell recording

Recordings were performed at 32 °C (TC-344B, Warner Instrument Corporation). VP neurons were visualized using an Olympus BX51WI microscope and recorded from using glass pipettes (1.3-2 MΩ, World Precision Instruments) filled with internal solution (in mM: 68 KCl, 65 D-gluconic acid potassium salt, 7.5 HEPES potassium, 1 EGTA, 1.25 MgCl2, 10 NaCl, 2.0 MgATP,

and 0.4 NaGTP, pH 7.2-7.3, 275mOsm). Excitatory synaptic transmission was blocked with CNQX (10 µM). Multiclamp 700B (Axon Instruments, Union City, CA) was used to record both membrane and action potentials and inhibitory postsynaptic currents (IPSCs) in whole cell configuration. Excitability and passive membrane properties were measured in current clamp mode while synaptic activity was measured in voltage clamp mode at a holding potential of -80 mV. Recordings were acquired at 10 kHz and filtered at 2 kHz using AxoGraph X software (AxoGraph Scientific, Sydney). To evoke IPSCs electrically, a bipolar stimulating electrode (FHC, Bowdoin, ME, USA) was placed ~200-300 µm anterior of the cell to maximize chances of stimulating NAc afferents. The stimulation intensity chosen evoked 30% to 70% of maximal IPSC. Recordings were collected every 10 seconds. Series resistance (Rs) measured with a −2 mV hyperpolarizing step (10 ms) given with each stimulus and holding current were always monitored online. Recordings with unstable Rs or when Rs exceeded 20 MΩ were aborted.

Excitability protocol

Excitability of neurons was evaluated in current clamp mode using eight 500 ms-long steps ranging from -60 mV to +80 mV (20 mV intervals). Inter-step interval was 3 seconds. The 8-step protocol was repeated 5 times with 3 s between repetitions. Only the depolarizing steps were used to determine excitability.

Long-term plasticity Measurements

Recorded in voltage clamp mode. Baseline evoked IPSCs were recorded at -80 mV for 3 min before applying the high-frequency stimulation (HFS) protocol. We used the same HFS protocol that was shown before to induce long term plasticity (depression) in inhibitory synapses in the VP (30, 47). The protocol consists of two trains separated by 20 s, each delivering 100 stimulations at 100 Hz. The amplitude of the pulses was identical to that inducing synaptic release when recording at -80 mV. During the HFS protocol, membrane potential was allowed to change freely (i.e. current clamp configuration). At the end of the second train of pulses recording was re-established at holding potential of -80 mV. Changes were measured at minutes 13-19 in all experiments, as after this time window the number of healthy recorded cells declined.

Figures

Figure 1

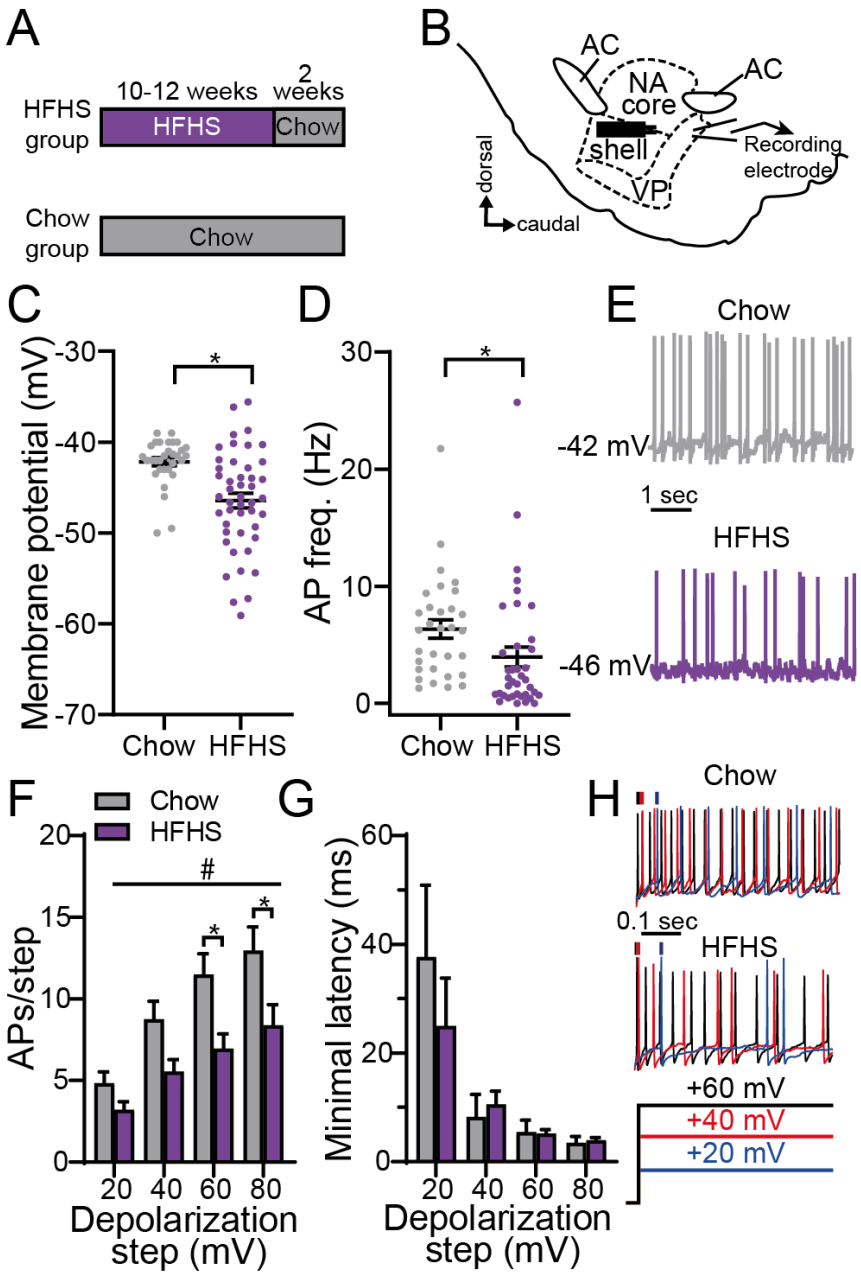

Figure 2

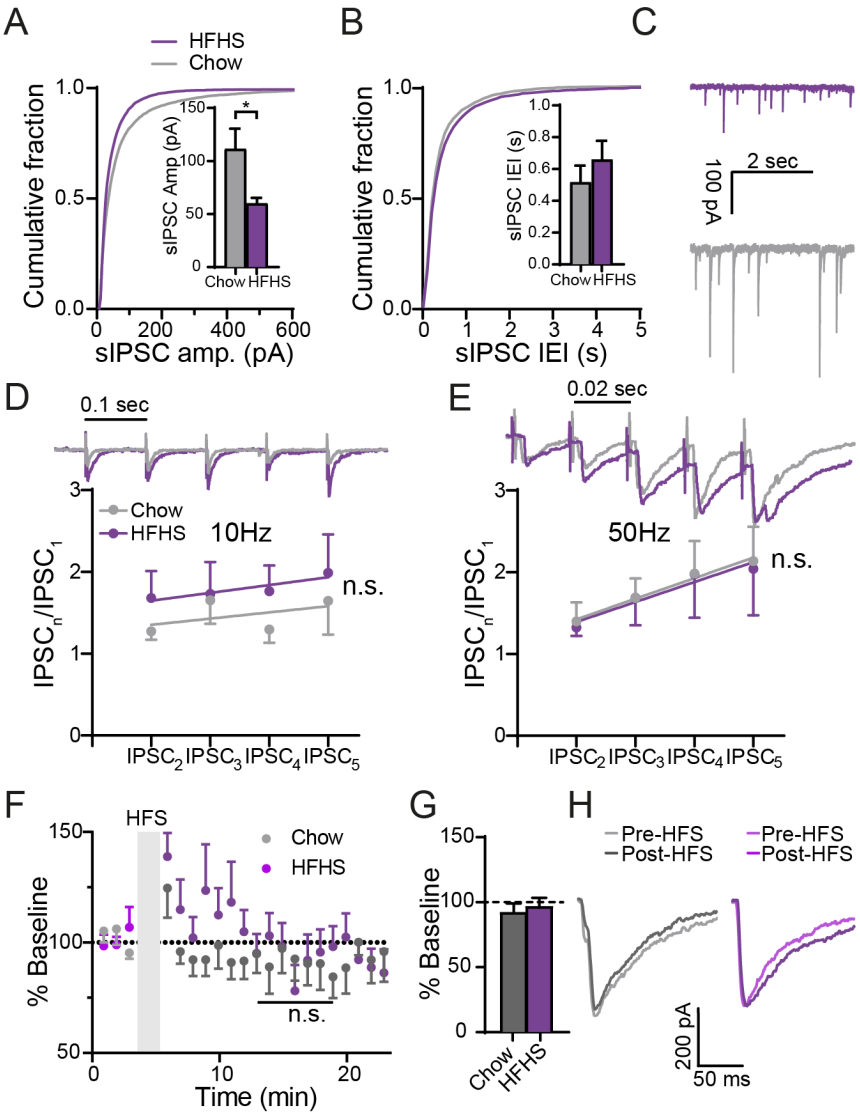
A
HFHS
Chow
Cumulative fraction
sIPSC amp. (pA)
sIPSC Amp (pA)
Chow HFHS
B
sIPSC IEI (s)
C
2 sec
100 pA
D
0.1 sec
Chow
HFHS
10Hz
n.s.
IPSC$_n$/IPSC$_1$
IPSC$_2$ IPSC$_3$ IPSC$_4$ IPSC$_5$
E
0.02 sec
50Hz
F
HFS
% Baseline
Time (min)
G
H
Pre-HFS
Post-HFS
200 pA
50 ms

Figure 3

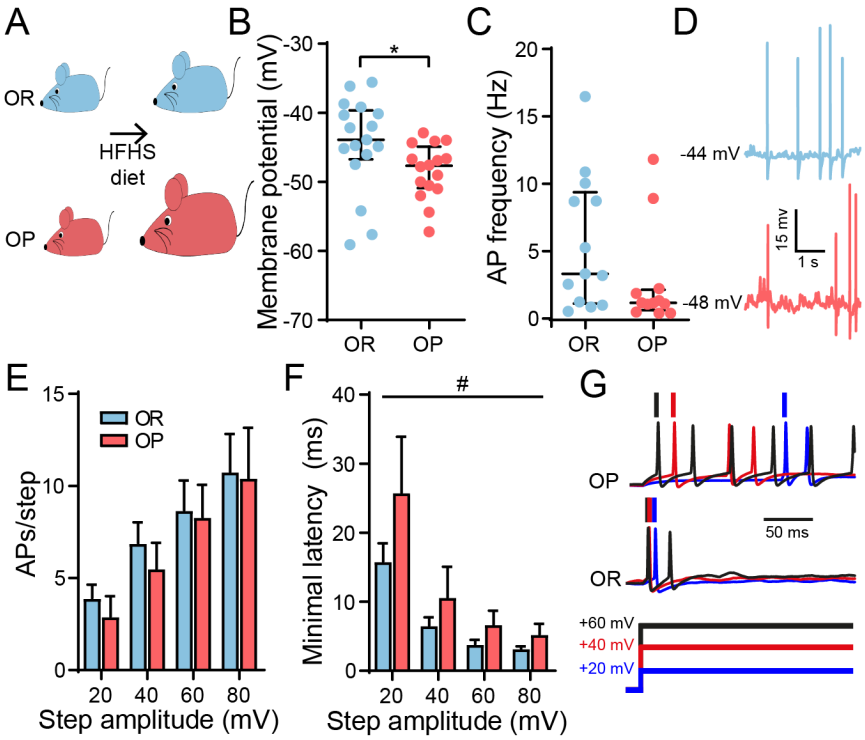
A
OR
HFHS
diet
OP
B
Membrane potential (mV)
-30
-40
-50
-60
-70
*
OR
OP
C
AP frequency (Hz)
20
15
10
5
0
OR
OP
D
-44 mV
-48 mV
15 mv
1 s
E
APs/step
15
10
5
0
OR
OP
20
40
60
80
Step amplitude (mV)
F
Minimal latency (ms)
40
30
20
10
0
#
20
40
60
80
Step amplitude (mV)
G
OP
OR
50 ms
+60 mV
+40 mV
+20 mV

Figure 4

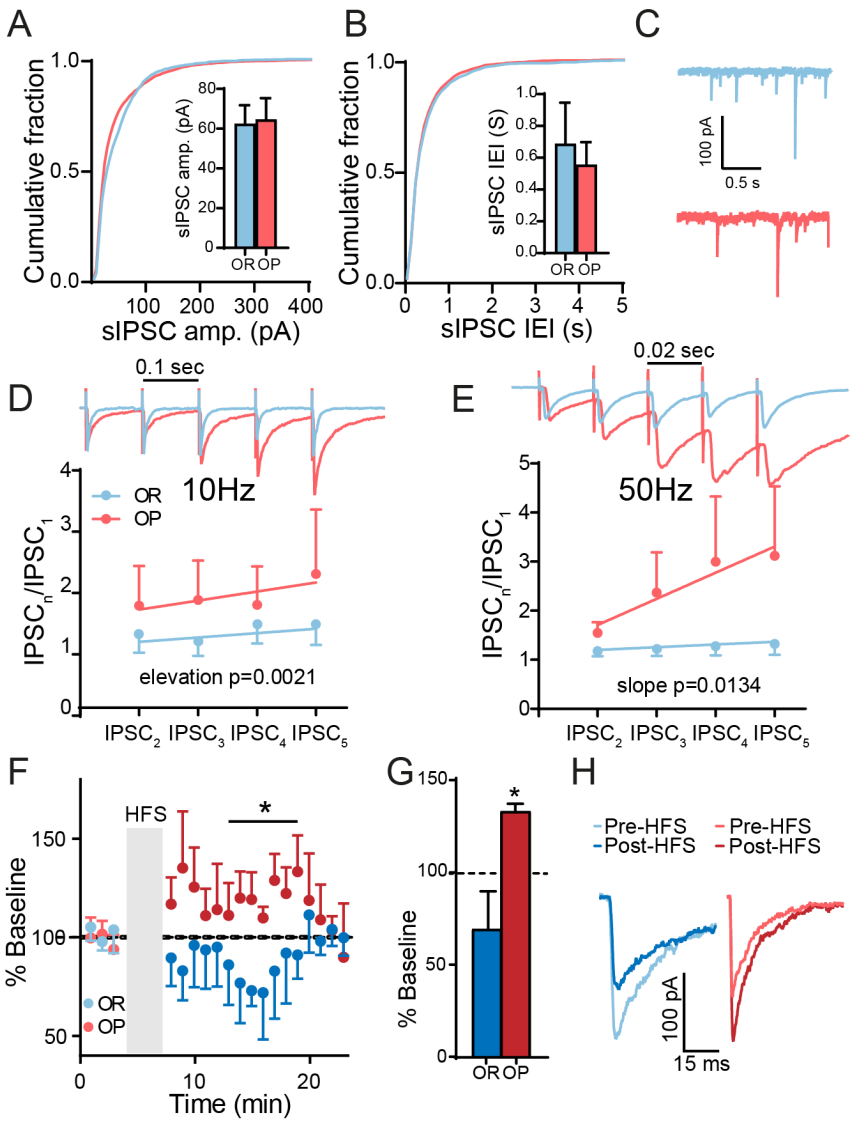
A
Cumulative fraction
sIPSC amp. (pA)
OR OP
B
sIPSC IEI (S)
sIPSC IEI (s)
C
100 pA
0.5 s
D
0.1 sec
10Hz
OR
OP
IPSC$_n$/IPSC$_1$
elevation p=0.0021
IPSC$_2$ IPSC$_3$ IPSC$_4$ IPSC$_5$
E
0.02 sec
50Hz
slope p=0.0134
F
HFS
% Baseline
Time (min)
G
H
Pre-HFS
Post-HFS
100 pA
15 ms

Figure 5

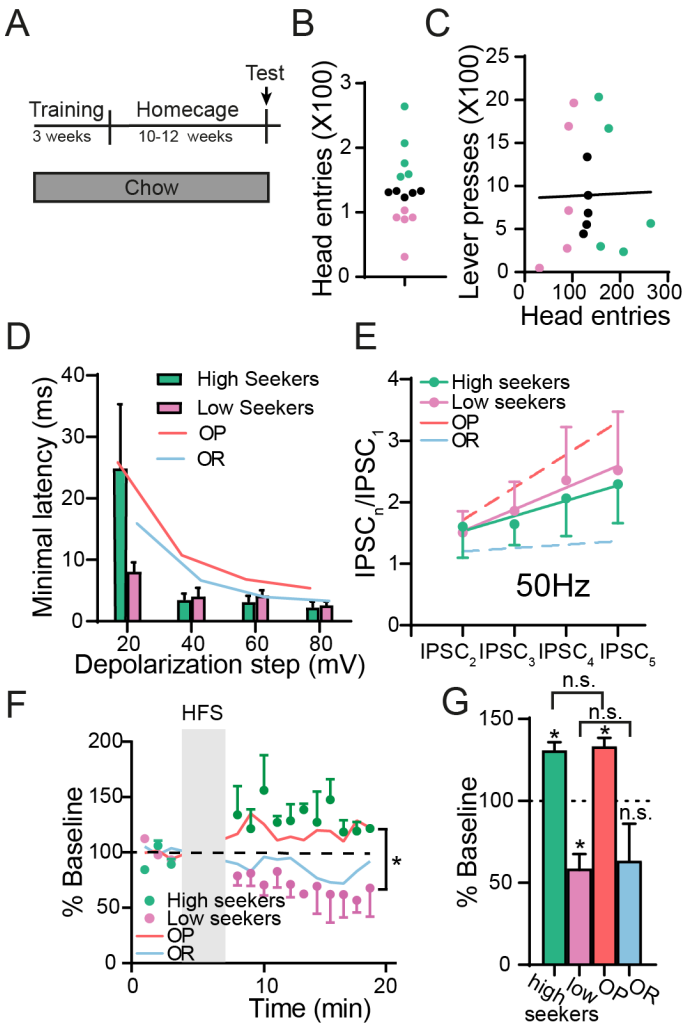
A
Training
3 weeks
Homecage
10-12 weeks
Test
Chow
B
Head entries (X100)
C
Lever presses (X100)
Head entries
D
Minimal latency (ms)
High Seekers
Low Seekers
OP
OR
Depolarization step (mV)
E
IPSC$_n$/IPSC$_1$
High seekers
Low seekers
OP
OR
50Hz
IPSC$_2$ IPSC$_3$ IPSC$_4$ IPSC$_5$
F
HFS
% Baseline
High seekers
Low seekers
OP
OR
Time (min)
G
% Baseline
n.s.
high seekers
low
OP
OR

Figure legend

Figure 1- Chronic High-Fat-High-Sugar diet diminishes excitability of ventral pallidum neurons

A) Experimental timeline. Mice in the HFHS group received a HFHS diet ad libitum in their home cages for 10-12 weeks and then returned to chow diet for 2 additional weeks before recordings. The control Chow group received regular chow during the same period of time. **B**) Recording setup. VP neurons were recorded from in sagittal slices while activating incoming axons using a bipolar stimulating electrode placed ~300 µm rostral to recorded cells. AC - anterior commissure; NA - nucleus accumbens. **C-E**) VP neurons of HFHS mice had lower membrane potential (**C**) (unpaired two-tailed t-test, * - $p<0.0001$) and lower action potential frequency (**D**) (unpaired two-tailed t-test, * - $p=0.048$) compared to chow mice. **E**) Representative current clamp traces. **F-H**) In a series of incrementing depolarizing steps the firing rate was significantly lower in VP neurons of HFHS mice compared with chow mice (**F**) (2-way ANOVA, # - main group effect $F_{(1,260)}=23.04$ $p<0.0001$, * - $p<0.01$ using Sidak's multiple comparisons test), but there was no difference in the minimal latency to the first action potential (**G**) (2-way ANOVA, main group effect $F_{(1,252)}=0.3689$ $p<0.5442$). **H**) Representative traces at 3 depolarizing steps. Data taken from 32-46 Cells in 14-21 mice per group.

Figure 2 - Chronic HFHS diet impairs spontaneous but not evoked GABA neurotransmission in the ventral pallidum

A) The cumulative probability plot of sIPSC amplitude is shifted to the left in HFHS mice, pointing to reduced sIPSC amplitude. Inset - median sIPSC amplitude was lower in HFHS mice (* - $p=0.0079$, Mann Whitney test) **B**) HFHS diet did not affect the IEI of sIPSCs in the VP. Inset - comparison of medians ($p=0.4062$, Mann Whitney test). **C**) Representative sIPSC traces. **D-E**) HFHS diet did not affect short term plasticity induced by 5 consecutive stimulations at 10 Hz (D) or 50 Hz (E) (10 Hz - 2-way ANCOVA, slope $F=0.00875$ $p=0.9257$, Elevation $F=1.949$ $p=0.1641$; 50 Hz - 2-way ANCOVA, slope $F=0.0009$ $p=0.9752$, Elevation $F=0.02642$ $p=0.8711$). **F-H**) The amplitude of evoked IPSCs after a high-frequency stimulation (HFS) did not differ between

chow and HFHS mice (Mixed effects ANOVA, main group effect $F_{(1,35)} = 0.016$ p=0.901, comparing minutes 13-19), or from baseline (one sample two-tailed t-test on minutes 13-19, Chow - t_{16}=0.96, p=0.35 HFHS - t_{19}=0.47, p=0.65). **H**) Representative traces. Data taken from 19-41 Cells in 13-24 mice per group.

Figure 3 – Obesity-prone mice show decreased excitability in the VP compared with obesity-resistant mice

A) After 10-12 weeks of chronic HFHS diet mice were split to obesity-resistant (OR, blue, bottom 33% of weight gainers) and obesity-prone (OP, red, top 33% of weight gainers). Middle 33% were not further analyzed. Full data on weight gain was published elsewhere (20). **B**) VP neurons of OP mice had lower membrane potential than those of OR mice (p<0.017, unpaired two-tailed t-test) **C**) There was no significant difference in the baseline firing frequency of VP neurons between OP and OR mice (p=0.0847 unpaired two-tailed t-test), although the average firing rate was lower in OP mice (mean ± SD, 5.20 ± 4.9 and 4.07 ± 7.4 for OR and OP mice, respectively) **D**) Representative traces. **E**) The firing rates during incrementing depolarization steps did not differ between OP and OR mice (2-way ANOVA, main group effect $F_{(1,84)}$ =0.4323, p=0.5126), but the minimal latency to the 1st action potential was longer in HFHS mice (2-way ANOVA, main group effect $F_{(1,78)}$ =5.37 p=0.0231), reflecting decreased excitability. **G**) Representative traces. Data taken from 9-16 cells in 7 mice per group.

Figure 4 - Obesity-prone and obesity-resistant mice show different synaptic plasticity in their evoked GABA input to the VP

A-C) sIPSC amplitude (A) and IEI (B) were similar between OP and OR mice. Insets - comparison of medians (Mann-Whitney test, p=0.87 for amplitude, p=0.90 for IEI). **C**) Representative sIPSC traces. **D-E**) Five consecutive pulses at 10 Hz (D) or 50 Hz (E) generated short-term potentiation of IPSCs in OP but not OR mice (10 Hz - 2-way ANCOVA, slope $F_{(1,4)}$=0.5546 p=0.4978, elevation $F_{(1,4)}$=34.32 P=0.0021; 50Hz – 2-way ANCOVA, slope $F_{(1,4)}$=17.78 p=0.0134). Insets - representative traces synchronized with x axis. **F-H**) HFS protocol given to inhibitory inputs to the VP generated LTP in OP mice but not in OR mice (Mixed effects ANOVA, main group effect

comparing minutes 13-19, $F_{(1,12)} = 5.29$ p=0.029; when comparing minutes 13-19 to 100% of baseline with one-sample two-tailed t-test - OP - t_5=4.25, p=0.008 OR - t_5=1.77, p=0.14). H) Representative traces. Data taken from 8-17 cells in 7 mice per group.

Figure 5 - Differences in long-term plasticity seen between OP and OR mice exist also between high and low food seekers without exposure to HFHS diet.

A) Experimental protocol. Mice were first trained to press a lever for 20 mg chow precision pellets in a progressive ratio paradigm. After 3 weeks of training mice were left in their home cages and fed on chow for 10-12 weeks. Then, the mice were tested again in the progressive ratio task and **B)** split according to the head entries into the food receptacle into "high seekers" (top third, green) and "low-seekers" (bottom third, pink). **C**) There was no correlation between the number of head entries and the number of active lever presses (R^2=0.0005385, slope not different than zero, p=0.9346). **D**) There was no difference in the minimal delay to the first action potential in a series of depolarizing steps (from +20 mV to + 80 mV) between high and low seekers (Two-way ANOVA, no main group effect, $F_{(1,47)}$=2.6, p=0.1135). **E**) There was no difference in short term plasticity between high and low seekers (5 stimulations at 50 Hz; Two-way ANCOVA , slope $F_{(1,4)}$=0.075 p=0.785, Elevation $F_{(1,4)}$=0.140 p=0.71). **F-G**) HFS protocol generated LTP in the high-seekers (green) and LTD in the low-seekers (pink) (Mixed effects ANOVA comparing minutes 13-19, main group effect $F_{(1,7)} = 19.14$ p= 0.003; comparing minutes 13-19 to 100% of baseline - one-sample Wilcoxon test, p<0.0001 and p<0.0001 for high and low seekers, respectively). These differences in long-term plasticity between high and low seekers were similar to the differences seen between OP (red line) and OR (blue line) mice after HFHS diet (Mixed effects ANOVA, no group effect when comparing OP mice to high-seekers ($F_{(1,9)}$=0.7375 p=0.4128) or OR mice to low-seekers ($F_{(1,12)}$=0.2007 p=0.6622); p>0.05 using two-tailed unpaired t-test when comparing minutes 13-19 in high seekers to OP mice or low seekers to OR mice). Data taken from 4-9 Cells in 5 mice per group.

Bibliography

1. OBESITY, OVERWEIGHT OBESITY and overweight.
2. Kenny PJ (2011): Reward mechanisms in obesity: new insights and future directions. *Neuron*. 69(4): 664–79.
3. de Macedo IC, de Freitas JS, da Silva Torres IL (2016): The influence of palatable diets in reward system activation: a mini review. *Adv. Pharmacol. Sci.* 2016: 7238679.
4. Stuber GD, Wise RA (2016): Lateral hypothalamic circuits for feeding and reward. *Nat. Neurosci.* 19(2): 198–205.
5. Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA, Wichmann R, *et al.* (2015): Decoding neural circuits that control compulsive sucrose seeking. *Cell*. 160(3): 528–41.
6. Friend DM, Devarakonda K, O'Neal TJ, Skirzewski M, Papazoglou I, Kaplan AR, *et al.* (2017): Basal ganglia dysfunction contributes to physical inactivity in obesity. *Cell Metab.* 25(2): 312–21.
7. de Weijer BA, van de Giessen E, van Amelsvoort TA, Boot E, Braak B, Janssen IM, *et al.* (2011): Lower striatal dopamine d2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res.* 1(1): 37.
8. Haltia LT, Rinne JO, Merisaari H, Maguire RP, Savontaus E, Helin S, *et al.* (2007): Effects of intravenous glucose on dopaminergic function in the human brain in vivo. *Synapse*. 61(9): 748–56.
9. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, *et al.* (2001): Brain dopamine and obesity. *Lancet*. 357(9253): 354–57.
10. Johnson PM, Kenny PJ (2010): Dopamine d2 receptors in addiction-like reward

dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* 13(5): 635–41.

11. Martire SI, Maniam J, South T, Holmes N, Westbrook RF, Morris MJ (2014): Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress. *Behav. Brain Res.* 265: 132–41.

12. Vucetic Z, Kimmel J, Reyes TM (2011): Chronic high-fat diet drives postnatal epigenetic regulation of μ-opioid receptor in the brain. *Neuropsychopharmacology*. 36(6): 1199–1206.

13. Alsiö J, Olszewski PK, Norbäck AH, Gunnarsson ZEA, Levine AS, Pickering C, *et al.* (2010): Dopamine d1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience*. 171(3): 779–87.

14. Brown RM, Kupchik YM, Spencer S, Garcia-Keller C, Spanswick DC, Lawrence AJ, *et al.* (2017): Addiction-like synaptic impairments in diet-induced obesity. *Biol. Psychiatry*. 81(9): 797–806.

15. Oginsky MF, Ferrario CR (2019): Eating “junk food” has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible versus -resistant rats. *J. Neurophysiol.* 122(3): 1264–73.

16. Gipson CD, Kupchik YM, Kalivas PW (2014): Rapid, transient synaptic plasticity in addiction. *Neuropharmacology*. 76 Pt B: 276–86.

17. Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, Kalivas PW (2013): Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation.

Neuron. 77(5): 867–72.

18. Gipson CD, Reissner KJ, Kupchik YM, Smith ACW, Stankeviciute N, Hensley-Simon ME, *et al.* (2013): Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proc Natl Acad Sci USA*. 110(22): 9124–29.

19. Derman RC, Ferrario CR (2018): Enhanced incentive motivation in obesity-prone rats is mediated by nac core cp-ampars. *Neuropharmacology*. 131: 326–36.

20. Inbar D, Gendelis S, Mesner S, Menahem S, Kupchik YM (2019): Chronic calorie-dense diet drives differences in motivated food seeking between obesity-prone and resistant mice. *Addict. Biol.*

21. Mancino S, Burokas A, Gutiérrez-Cuesta J, Gutiérrez-Martos M, Martín-García E, Pucci M, *et al.* (2015): Epigenetic and proteomic expression changes promoted by eating addictive-like behavior. *Neuropsychopharmacology*. 40(12): 2788–2800.

22. Tripathi A, Prensa L, Mengual E (2013): Axonal branching patterns of ventral pallidal neurons in the rat. *Brain Struct. Funct.* 218(5): 1133–57.

23. Stratford TR, Kelley AE, Simansky KJ (1999): Blockade of gabaa receptors in the medial ventral pallidum elicits feeding in satiated rats. *Brain Res.* 825(1–2): 199–203.

24. Zahm DS, Heimer L (1990): Two transpallidal pathways originating in the rat nucleus accumbens. *J. Comp. Neurol.* 302(3): 437–46.

25. Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, Kalivas PW (2015): Coding the direct/indirect pathways by d1 and d2 receptors is not valid for accumbens projections. *Nat. Neurosci.* 18(9): 1230–32.

26. Root DH, Melendez RI, Zaborszky L, Napier TC (2015): The ventral pallidum: subregion-

specific functional anatomy and roles in motivated behaviors. *Prog. Neurobiol.* 130: 29–70.

27. Cromwell HC, Berridge KC (1993): Where does damage lead to enhanced food aversion: the ventral pallidum/substantia innominata or lateral hypothalamus? *Brain Res.* 624(1–2): 1–10.

28. Shimura T, Imaoka H, Yamamoto T (2006): Neurochemical modulation of ingestive behavior in the ventral pallidum. *Eur. J. Neurosci.* 23(6): 1596–1604.

29. Smith KS, Tindell AJ, Aldridge JW, Berridge KC (2009): Ventral pallidum roles in reward and motivation. *Behav. Brain Res.* 196(2): 155–67.

30. Kupchik YM, Scofield MD, Rice KC, Cheng K, Roques BP, Kalivas PW (2014): Cocaine dysregulates opioid gating of gaba neurotransmission in the ventral pallidum. *J. Neurosci.* 34(3): 1057–66.

31. Alexander RPD, Mitry J, Sareen V, Khadra A, Bowie D (2019): Cerebellar stellate cell excitability is coordinated by shifts in the gating behavior of voltage-gated na+ and a-type k+ channels. *Eneuro*. 6(3):

32. Zhu C, Yao Y, Xiong Y, Cheng M, Chen J, Zhao R, *et al.* (2017): Somatostatin neurons in the basal forebrain promote high-calorie food intake. *Cell Rep.* 20(1): 112–23.

33. Smith KS, Berridge KC (2005): The ventral pallidum and hedonic reward: neurochemical maps of sucrose “liking” and food intake. *J. Neurosci.* 25(38): 8637–49.

34. Brownell KD, Walsh BT, eds (2018): *Eating Disorders and Obesity, Third Edition: A Comprehensive Handbook*, illustrated, ed. Guilford Publications,

35. Burger KS, Stice E (2014): Greater striatopallidal adaptive coding during cue-reward

learning and food reward habituation predict future weight gain. *Neuroimage*. 99: 122–28.

36. Haahr ME, Rasmussen PM, Madsen K, Marner L, Ratner C, Gillings N, *et al.* (2012): Obesity is associated with high serotonin 4 receptor availability in the brain reward circuitry. *Neuroimage*. 61(4): 884–88.

37. Hamer M, Batty GD (2019): Association of body mass index and waist-to-hip ratio with brain structure: uk biobank study. *Neurology*. 92(6): e594–600.

38. Baek K, Morris LS, Kundu P, Voon V (2017): Disrupted resting-state brain network properties in obesity: decreased global and putaminal cortico-striatal network efficiency. *Psychol. Med.* 47(4): 585–96.

39. Abraham WC, Bear MF (1996): Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci.* 19(4): 126–30.

40. Kombian SB, Malenka RC (1994): Simultaneous ltp of non-nmda- and ltd of nmda-receptor-mediated responses in the nucleus accumbens. *Nature*. 368(6468): 242–46.

41. Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001): Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat. Neurosci.* 4(12): 1217–23.

42. Martin M, Chen BT, Hopf FW, Bowers MS, Bonci A (2006): Cocaine self-administration selectively abolishes ltd in the core of the nucleus accumbens. *Nat. Neurosci.* 9(7): 868–69.

43. Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, *et al.* (2010): Transition to addiction is associated with a persistent impairment in synaptic

plasticity. *Science*. 328(5986): 1709–12.

44. Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A, *et al.* (2009): N-acetylcysteine reverses cocaine-induced metaplasticity. *Nat. Neurosci.* 12(2): 182–89.

45. Inbar K, Levi LA, Bernat N, Odesser T, Inbar D, Kupchik YM (2019): Cocaine dysregulates dynorphin modulation of inhibitory neurotransmission in the ventral pallidum in a cell-type-specific manner. *J. Neurosci.*

46. Levi LA, Inbar K, Nachshon N, Bernat N, Gatterer A, Inbar D, *et al.* (2019): Projection-specific potentiation of ventral pallidal glutamatergic outputs after abstinence from cocaine. *J. Neurosci.*

47. Heinsbroek JA, Neuhofer DN, Griffin WC, Siegel GS, Bobadilla A-C, Kupchik YM, *et al.* (2017): Loss of plasticity in the d2-accumbens pallidal pathway promotes cocaine seeking. *J. Neurosci.* 37(4): 757–67.