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Abstract

We aim to identify physiologic regulators of dopamine (DA) signaling in obesity but previously did not find a compelling relationship with insulin sensitivity measured by oral-minimal model (OMM) and DA subtype 2 and 3 receptor (D2/3R) binding potential (BP_{ND}). Reduced disposition index (DI), a β-cell function metric that can also be calculated by OMM, was shown to predict a negative reward behavior that occurs in states of lower endogenous DA. We hypothesized that reduced DI would occur with higher D2/3R BP_{ND}, reflecting lower endogenous DA. Participants completed PET scanning, with a displaceable radioligand to measure D2/3R BP_{ND}, and a 5-hour oral glucose tolerance test to measure DI by OMM. We studied 26 age-similar females without (n = 8) and with obesity (n = 18) (22 vs 39 kg/m^2). Reduced DI predicted increased striatal D2/3R BP_{ND} independent of BMI. By accounting for β-cell function, we were able to determine that the state of insulin and glucose metabolism is pertinent to striatal D2/3R BP_{ND} in obesity.

Clinical Trial Registration Number: NCT00802204

Introduction

Diminished dopamine (DA) signaling and food reward are associated with obesity and are postulated to contribute to and/or perpetuate obesity. The mechanisms of the anorexic effects of insulin include regulating food reward through DA signaling, and thus, impaired insulin sensitivity (i.e. insulin resistance) is expected to dysregulate DA signaling [1] as occurs in rodent models of diet induced obesity (DIO) [2]. We previously reported that higher body mass index (BMI) and lower fasting acyl ghrelin concentrations were associated with increased striatal DA subtype 2 and 3 receptor (D2/3R) binding potential (BP_{ND}), which we interpreted to reflect lower levels of endogenous DA competing with the displaceable radioligand, [18F] fallypride [3]. Reduced striatal DA levels occur in DIO rodents [4] and one human report had trend level data demonstrating reduced pharmacologically-induced DA release in obesity [5]. Using the same radioligand we utilized, [18F]fallypride, others found positive relationships

between BMI and D2/3R BP_{ND} in the dorsal striatum (caudate and putamen) with conflicting findings in the ventral striatum [6, 7]. In our previous report, insulin resistance also predicted higher striatal D2/3R binding, (i.e. lower endogenous DA), but this effect was not independent of BMI [3]. Eisenstein et al used β-cell function and [^{11}C](N-methyl)benperidol([^{11}C]NMB), a non-displaceable, D2 receptor-selective radioligand, to examine the relationship between DA signaling, obesity, and insulin. They did not find associations between striatal D2R levels and BMI or β-cell function (determined by disposition index, DI). This lack of relationship between BMI and receptor levels with a non-displaceable radioligand supported our interpretation that differences in endogenous DA levels were a predominate factor defining the relationship we identified with BMI and receptor levels measured with a displaceable radioligand. Eisenstein et al did report that β-cell function was associated with increased delayed discounting, a detrimental reward behavior which reflects impaired inhibitory control and is attenuated by agents that increase extracellular DA levels. Essentially, they found that impaired β-cell function occurred with a behavior that is present in a state of decreased endogenous DA [8]. This finding prompted us to re-examine our data to determine the relationship of β-cell function measured by DI to striatal D2/3R BP_{ND} estimated with a displaceable radioligand. Further we sought to determine if any identified relationships were independent of BMI as our primary aim is to define physiologic regulators of DA signaling.

Methods

The study protocol was approved by the Vanderbilt University Institutional Review Board and all participants gave written informed consent. We studied 26 weight-stable females, 8 non-obese (22±3 kg/m^2) and 18 obese (39±6 kg/m^2) of similar age (Table 1), 22 who were included in our prior report [3]. Screening included history and physical exam, laboratory testing including urine drug screen, and magnetic resonance imaging (MRI) of the brain. Exclusion included pregnancy, significant current psychiatric, neurologic or medical condition. One participant had diet-controlled type 2 diabetes mellitus. Individuals were also excluded if current tobacco use, substance abuse or heavy alcohol use, or if treated with central acting medications or insulin sensitizing agents in the preceding six months.

As detailed previously [3], before admission to the Vanderbilt University Clinical Research Center (CRC) participants were requested to refrain from exercise, alcohol and excess caffeine for 48 hours. On the day of admission, at ~18:30h after an eight-hour fast, blood was collected (with serine protease inhibitor and subsequent plasma acidification for acyl ghrelin measurement) then positron emission tomography (PET) scanning with [^{18}F]fallypride was completed. Participants stayed overnight at the CRC and the next morning underwent a five-hour 75

Table 1. Total cohort of participants that completed baseline PET imaging and OGTT.

	Non-obese (n = 8)	Obese (n = 18)	p-value
Weight (kg)	59±7	106±17	
BMI (kg/m^2)	22±3	39±6	
Age (y)	41±9	39±8	0.489
SI (10^{-4} * min^{-1} *μU^{-1} * mL)	11.2±4.1	3.9±2.5	<0.001
ϕ_{total} (10^9 min-1)	26.7±8.6	30.8±10.3	0.336
DI (10^6 min^{-2} *μU^{-1} * mL)	29.6±13.7	10.8±6.67	0.005
Regional D2/3R BP_{ND}			
Caudate	28.9±3.3	32.6±2.8	0.006
Putamen	34.2±3.8	37.6±2.5	0.013
Ventral Striatum	19.1±3.8	22.1±2.6	0.030

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gram oral glucose tolerance test (OGTT) with 11 blood draws for glucose, insulin and C-peptide measurement [9]. The oral-minimal model (OMM) was applied to provide estimates of insulin sensitivity (SI) and insulin secretion (ϕ_{total}) by modeling the relationships of glucose with insulin and C-peptide with glucose, respectively [9]. The disposition index (DI) is the product of SI and $\phi\phi_{total}$ and describes the β-cells' ability to respond to a decrease in insulin sensitivity by appropriately increasing insulin secretion [10].

Imaging techniques and analysis were also completed as previously detailed [3]. Briefly, T1 weighted images of the brain were obtained on either a 1.5T or 3T MRI at screening. PET scanning was initiated with a bolus injection of [18F]fallypride and was completed in three scan sequences over approximately 3.5 hours. For imaging analysis, the serial PET scans were co-registered to each other and to the thin section T1-weighted MRI scans using a mutual information rigid body algorithm. The full reference region method was used to calculate regional DA D2/D3R BP$_{ND}$ with the cerebellum as the reference region. Region of interest (ROI) analysis of the caudate, putamen, and ventral striatum was used due to the regions' relevance to reward behaviors and being the most consistently reported ROIs in the literature. The caudate and putamen were manually drawn on axial slices of the MRI at approximately 2–12 mm above the anterior commissure—posterior commissure line. The ventral striatum was delineated on coronal slices using established criteria [11]. The ROIs were delineated bilaterally on the MRI images and transferred to the coregistered PET scans. The average value between the two hemispheres for each region was used for analysis.

Data are presented as mean ± standard deviation (SD) and non-obese vs obese, respectively. Demographic and outcome measures were compared using student t-tests. The relationship between striatal D2/3R BP$_{ND}$ and age, BMI, SI, DI, ϕ_{total} were evaluated by linear regression. The β's reported are the unstandardized coefficients.

Results

D2/3R BP$_{ND}$ was 10–15% higher in the obese (caudate 29 vs. 33, p = 0.006; putamen 34 vs. 38, p = 0.013; ventral striatum 19 vs. 22, p = 0.030) (Table 1). We again found that higher BMI, lower insulin sensitivity (SI), and lower fasting acyl ghrelin levels predicted increased D2/3R binding throughout the striatum (Table 2, Fig 1). DI also had significant negative relationships with striatal D2/3R BP$_{ND}$. Neither age (p-values = 0.11–0.60) nor ϕ_{total} (p-values = 0.42–0.98) were associated with regional receptor binding in our cohort. When adjusted for BMI, SI did not maintain relationships with D2/3R BP$_{ND}$ (p-values = 0.1–0.2) but DI maintained robust negative relationships with D2/3R BP$_{ND}$ (Table 3). DI explained all variance in D2/3R BP$_{ND}$ attributed to BMI without significantly modifying parameter estimates for DI. Since in our previous report fasting acyl ghrelin concentrations were associated with striatal D2/3R BP$_{ND}$ independent of BMI, we explored the relationship of both DI and fasting acyl ghrelin concentrations to receptor binding. When both were included as covariates to explain striatal D2/3R BP$_{ND}$, DI continued to explain the majority of the variance (p values = 0.088–0.028) while acyl ghrelin concentrations did not maintain independent (p values ≥ 0.15) relationships with

Table 2. Univariate metabolic predictors of striatal D2/3R binding potential.

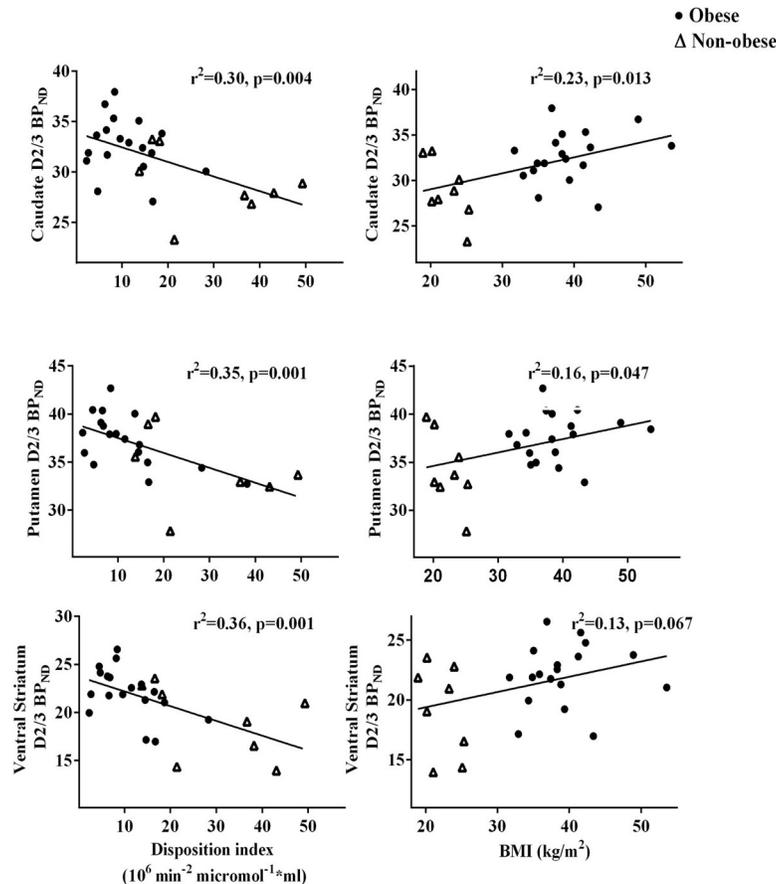| Region	BMI			SI			Acyl ghrelin			DI		
	R	β	p-value	R	β	p-value	R	β	p-value	R	β	p-value
Caudate	0.482	0.18	0.013	0.485	-0.39	0.012	0.511	-0.016	0.008	0.545	-0.14	0.004
Putamen	0.394	0.14	0.047	0.492	-0.36	0.011	0.549	-0.017	0.004	0.595	-0.15	0.001
Ventral Striatum	0.364	0.13	0.067	0.477	-0.34	0.014	0.491	-0.015	0.011	0.601	-0.15	0.001

Fig 1. Linear regressions for striatal D2/3R availability with disposition index and D2/3R availability with BMI.

striatal D2/3R BP$_{ND}$; however, the parameter estimates for DI changed by 19–31%, demonstrating acyl ghrelin concentrations as a relevant modifier (Table 3).

Discussion

We determined that impaired β-cell function (lower DI) is associated with higher striatal D2/3R binding. Again, this is interpreted as lower endogenous DA as we used a displaceable radioligand (Fig 2). DI allows insulin secretion to be interpreted in light of the prevailing insulin sensitivity, providing a quantitative index that estimates an individual's state of glucose tolerance based on a physiologic challenge [12]. Our regressions demonstrate that this physiologic measure can describe over twice the variance in D2/3R binding as the non-physiologic BMI

Table 3. Multivariate relationships for striatal D2/3R binding potential with either DI and BMI or DI and fasting acyl ghrelin concentrations.

	Caudate			Putamen			Ventral Striatum		
	R	**β**	**p-value**	**R**	**β**	**p-value**	**R**	**β**	**p-value**
DI	0.589	-0.11	0.057	0.601	-0.14	0.012	0.602	-0.15	0.008
BMI		0.10	0.201		0.04	0.618		0.02	0.788
DI	0.592	-0.10	0.088	0.642	-0.11	0.049	0.623	-0.12	0.028
Acyl ghrelin		-0.009	0.184		-0.009	0.145		-0.006	0.323
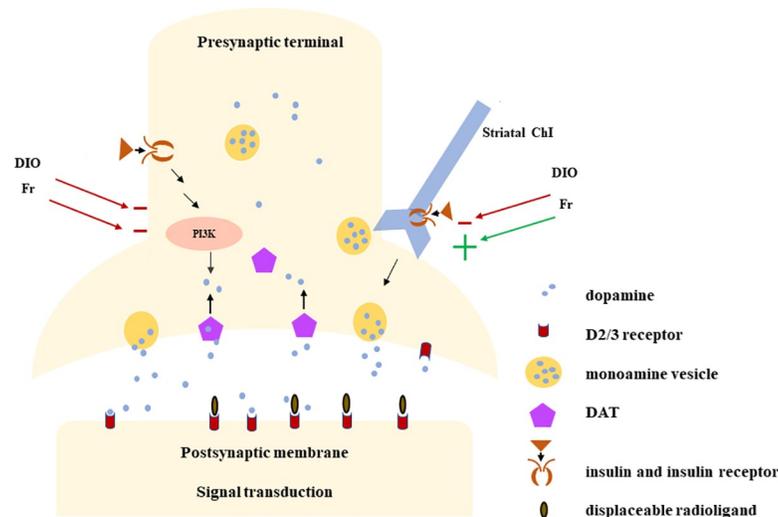
Fig 2. Presynaptic terminal: Simplified schema of insulin effects on DA neuron synapse in the striatum. Evidence supports cell surface DAT and striatal ChI excitability vary by diet-induced effects on both insulin secretion/levels and insulin sensitivity. Postsynaptic membrane: with PET imaging the displaceable radioligand competes with extracellular DA for binding.

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measure. The effect size we detail for DI with D2/3R binding is substantially greater than what others have reported for BMI with D2/3R binding using the same radioligand [7]. We did not find age to be a modifier of striatal D2/3R binding as others have reported; however it is important to note the DI declines with aging and can be modified by weight loss [13]. BMI is commonly used to explore the effect of obesity on central reward signaling, but the current work demonstrates its limitations. The state of insulin and glucose metabolism are pertinent to design and interpretation of studies of DA signaling in obesity. Further, if aspects of insulin and glucose metabolism are influencing central reward signaling, this presents the potential for personalized therapeutic targets in diseases of reward signaling and obesity.

Besides peripherally secreted insulin directly modifying DA signaling there is evidence for a dopaminergic gut-brain axis at least partially mediated via the vagus. There are hypothalamic-vagal-pancreatic connections with dopaminergic innervations[14] and peripheral insulin also modifies vagal afferents[15]. Intact vagal transmission is necessary for meal-induced increases in striatal DA release[16], however it is unknown if this is modified by insulin signaling. Parkinson's Disease, a state of central dopaminergic depletion, occurs with impaired vagal activity and altered glucose-stimulated insulin secretion (GSIS) [17]. In rodents, depleting nigrostriatal DA alters colonic DA, D2R and DA type 1 receptor levels which is hypothesized to contribute to the gastrointestinal symptoms of Parkinson's Disease [18]. We are not aware of any direct evidence that central DA depletion influences pancreatic β-cell DA signaling however, insulin sensitivity increases with acute deep brain stimulation (DBS) of the internal capsule, (which is known to increase striatal DA release) and decreases with acute pharmacologic depletion of extracellular DA [19]. At the level of the DA neuron (Fig 2), insulin increases cell-surface expression of the DA transporter (DAT) which takes DA back up into the presynaptic neuron. This function is impaired in insulin-deficient streptozotocin (STZ) diabetic rodents[20] and in insulin resistant (impaired insulin signaling) diet induced obese (DIO)[15] as both have reduced cell-surface DAT. DAT activity is also reduced in food restricted (Fr) animals[21] that have low insulin levels. While direct insulin exposure in the ventral tegmental area (VTA) causes reduced extracellular DA levels via increased DAT activity [22], Stouffer et al reported

that direct insulin exposure in the striatum has competing effects on extracellular DA. In the striatum, insulin does promote DA reuptake via DAT but it also increases the excitability of striatal cholinergic interneurons (ChI) causing release of DA which is the predominant effect. As this is mediated by insulin receptors on ChI, it is modified by insulin sensitivity such that insulin resistant DIO obese and insulin sensitive food-restricted rodents have attenuated and amplified DA release, respectively[21]. This demonstrates that both insulin secretion/levels and insulin sensitivity influence DA signal transduction consistent with our current report.

We hypothesize that at least some of the inconsistencies in the literature related to DA signaling in obesity (reviewed by Dang, L et al) [7] are due to metabolic variation within obese cohorts. Other specifics of our protocol including weight stability, restraining from exercise [23] before imaging, fasting pre-scan [24], and evening scan times [25] are also posited to contribute to our findings as pre-clinical studies support these as relevant modifiers of DA signaling. The modification of the relationship between DI and D2/3R binding by ghrelin support both the relevance of fasting, when ghrelin reaches its peak, and the known effects of ghrelin on DA mediated reward signaling [1]. A more comprehensive understanding as to how weight gain modifies the dopaminergic gut/pancreatic-brain axis will be relevant to various disease processes including dysregulated reward signaling in obesity.

Supporting information

S1 File.
(DOCX)

Acknowledgments

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Writing – review & editing: Julia P. Dunn, Naji N. Abumrad, Bruce W. Patterson, Robert M. Kessler, Robyn A. Tamboli.

References

1. Murray S, Tulloch A, Gold MS, Avena NM. Hormonal and neural mechanisms of food reward, eating behaviour and obesity. Nat Rev Endocrinol. 2014; 10(9):540–52. https://doi.org/10.1038/nrendo.2014.91 PMID: 24958311.

2. Speed N, Saunders C, Davis AR, Owens WA, Matthies HJ, Saadat S, et al. Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. PLoS One. 2011; 6(9):e25169. https://doi.org/10.1371/journal.pone.0025169 PMID: 21969871; PubMed Central PMCID: PMCPMC3182178.

3. Dunn JP, Kessler RM, Feurer ID, Volkow ND, Patterson BW, Ansari MS, et al. Relationship of dopamine type 2 receptor binding potential with fasting neuroendocrine hormones and insulin sensitivity in human obesity. Diabetes care. 2012; 35(5):1105–11. https://doi.org/10.2337/dc11-2250 PMID: 22432117; PubMed Central PMCID: PMC3329842.

4. Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience. 2009; 159(4):1193–9. https://doi.org/10.1016/j.neuroscience.2009.02.007 PMID: 19409204

5. van de Giessen E, Celik F, Schweitzer DH, van den Brink W, Booij J. Dopamine D2/3 receptor availability and amphetamine-induced dopamine release in obesity. J Psychopharmacol. 2014; 28(9):866–73. https://doi.org/10.1177/0269881114531664 PMID: 24785761.

6. Guo J, Simmons WK, Herscovitch P, Martin A, Hall KD. Striatal dopamine D2-like receptor correlation patterns with human obesity and opportunistic eating behavior. Mol Psychiatry. 2014; 19(10):1078–84. Epub 2014/09/10. https://doi.org/10.1038/mp.2014.102 PMID: 25199919; PubMed Central PMCID: PMCPMC4189966.

7. Dang LC, Samanez-Larkin GR, Castrellon JJ, Perkins SF, Cowan RL, Zald DH. Associations between dopamine D2 receptor availability and BMI depend on age. NeuroImage. 2016; 138:176–83. Epub 2016/05/22. https://doi.org/10.1016/j.neuroimage.2016.05.044 PMID: 27208860; PubMed Central PMCID: PMCPMC4927378.

8. Eisenstein SA, Gredysa DM, Antenor-Dorsey JA, Green L, Arbelaez AM, Koller JM, et al. Insulin, Central Dopamine D2 Receptors, and Monetary Reward Discounting in Obesity. PLoS One. 2015; 10(7):e0133621. Epub 2015/07/21. https://doi.org/10.1371/journal.pone.0133621 PMID: 26192187; PubMed Central PMCID: PMCPMC4507849.

9. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. Diabetes. 2001; 50(1):150–8. Epub 2001/01/09. PMID: 11147781.

10. Dalla Man C, Yarasheski KE, Caumo A, Robertson H, Toffolo G, Polonsky KS, et al. Insulin sensitivity by oral glucose minimal models: validation against clamp. American journal of physiology. 2005; 289 (6):E954–9. https://doi.org/10.1152/ajpendo.00076.2005 PMID: 16014353.

11. Mawlawi O, Martinez D, Slifstein M, Broft A, Chatterjee R, Hwang DR, et al. Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D(2) receptor parameter measurements in ventral striatum. J Cereb Blood Flow Metab. 2001; 21(9):1034–57. Epub 2001/08/29. https://doi.org/10.1097/00004647-200109000-00002 PMID: 11524609.

12. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R. The oral minimal model method. Diabetes. 2014; 63(4):1203–13. https://doi.org/10.2337/db13-1198 PMID: 24651807; PubMed Central PMCID: PMCPMC4179313.

13. Villareal DT, Banks MR, Patterson BW, Polonsky KS, Klein S. Weight loss therapy improves pancreatic endocrine function in obese older adults. Obesity (Silver Spring, Md. 2008; 16(6):1349–54. https://doi.org/10.1038/oby.2008.226 PMID: 18388888; PubMed Central PMCID: PMCPMC2799929.

14. Lopez Vicchi F, Luque GM, Brie B, Nogueira JP, Garcia Tornadu I, Becu-Villalobos D. Dopaminergic drugs in type 2 diabetes and glucose homeostasis. Pharmacological research. 2016; 109:74–80. Epub 2016/01/10. https://doi.org/10.1016/j.phrs.2015.12.029 PMID: 26748034.

15. Warne JP, Horneman HF, Ginsberg AB, Pecoraro NC, Foster MT, Akana SF, et al. Mapping brain c-Fos immunoreactivity after insulin-induced voluntary lard intake: insulin- and lard-associated patterns. J Neuroendocrinol. 2007; 19(10):794–808. https://doi.org/10.1111/j.1365-2826.2007.01593.x PMID: 17850462.

16. Tellez LA, Medina S, Han W, Ferreira JG, Licona-Limon P, Ren X, et al. A gut lipid messenger links excess dietary fat to dopamine deficiency. Science (New York, NY. 2013; 341(6147):800–2. https://doi.org/10.1126/science.1239275 PMID: 23950538.

17. Knudsen K, Hartmann B, Fedorova TD, Ostergaard K, Krogh K, Moller N, et al. Pancreatic Polypeptide in Parkinson's Disease: A Potential Marker of Parasympathetic Denervation. Journal of Parkinson's disease. 2017; 7(4):645–52. Epub 2017/09/19. https://doi.org/10.3233/JPD-171189 PMID: 28922171.

18. Garrido-Gil P, Rodriguez-Perez AI, Dominguez-Meijide A, Guerra MJ, Labandeira-Garcia JL. Bidirectional Neural Interaction Between Central Dopaminergic and Gut Lesions in Parkinson's Disease Models. Molecular neurobiology. 2018; 55(9):7297–316. Epub 2018/02/07. https://doi.org/10.1007/s12035-018-0937-8 PMID: 29404956.

19. Ter Horst KW, Lammers NM, Trinko R, Opland DM, Figee M, Ackermans MT, et al. Striatal dopamine regulates systemic glucose metabolism in humans and mice. Sci Transl Med. 2018; 10(442). https://doi.org/10.1126/scitranslmed.aar3752 PMID: 29794060.

20. Williams JM, Owens WA, Turner GH, Saunders C, Dipace C, Blakely RD, et al. Hypoinsulinemia Regulates Amphetamine-Induced Reverse Transport of Dopamine. PLoS Biol. 2007; 5(10):e274. https://doi.org/10.1371/journal.pbio.0050274 PMID: 17941718

21. Stouffer MA, Woods CA, Patel JC, Lee CR, Witkovsky P, Bao L, et al. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. Nat Commun. 2015; 6:8543. https://doi.org/10.1038/ncomms9543 PMID: 26503322; PubMed Central PMCID: PMCPMC4624275.

22. Mebel DM, Wong JC, Dong YJ, Borgland SL. Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. Eur J Neurosci. 2012; 36 (3):2336–46. https://doi.org/10.1111/j.1460-9568.2012.08168.x PMID: 22712725; PubMed Central PMCID: PMCPMC5239666.

23. Lynch WJ, Peterson AB, Sanchez V, Abel J, Smith MA. Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis. Neuroscience and biobehavioral reviews. 2013; 37(8):1622–44. https://doi.org/10.1016/j.neubiorev.2013.06.011 PMID: 23806439; PubMed Central PMCID: PMCPMC3788047.

24. Roseberry AG. Acute fasting increases somatodendritic dopamine release in the ventral tegmental area. J Neurophysiol. 2015; 114(2):1072–82. Epub 2015/06/19. https://doi.org/10.1152/jn.01008.2014 PMID: 26084913; PubMed Central PMCID: PMCPMC4725115.

25. Webb IC, Baltazar RM, Wang X, Pitchers KK, Coolen LM, Lehman MN. Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. J Biol Rhythms. 2009; 24(6):465–76. https://doi.org/10.1177/0748730409346657 PMID: 19926806.