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Suboptimal Maternal Diets Alter mu Opioid Receptor and Dopamine Type 1 Receptor Binding but Exert no Effect on Dopamine Transporters in the Offspring Brain

Panayotis K. Thanos¹, Jianmin Zhuo¹, Lisa Robison¹, Ronald Kim¹, Mala Ananth¹, Ilon Choi¹, Adam Grunseich¹, Nicola M. Grissom³, Robert George², Foteini Delis¹, and Teresa M. Reyes³

¹Behavioral Neuropharmacology and Neuroimaging Laboratory, Research Institute on Addictions, University at Buffalo 14203, USA

²Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

³ Department of Psychiatry and Behavioral Neurosciences, University of Cincinnati, Cincinnati, OH 45237

Abstract

Birthweight is a marker for suboptimal fetal growth and development *in utero*. Offspring can be born large for gestational age (LGA), which is linked to maternal obesity or excessive gestational weight gain, as well as small for gestational age (SGA), arising from nutrient or calorie deficiency, placental dysfunction, or other maternal conditions (hypertension, infection). In humans, LGA and SGA babies are at an increased risk for certain neurodevelopmental disorders, including Attention Deficit /Hyperactivity Disorder, schizophrenia, and social and mood disorders. Using mouse models of LGA (maternal high fat (HF) diet) and SGA (maternal low protein (LP) diet) offspring, our lab has previously shown that these offspring display alterations in the expression of mesocorticolimbic genes that regulate dopamine and opioid function, thus indicating that these brain regions and neurotransmitter systems are vulnerable to gestational insults. Interestingly, these two maternal diets affected dopamine and opioid systems in somewhat opposing directions (e.g., LP offspring are generally hyperdopaminergic with reduced opioid expression, and the reverse is found for the HF offspring). These data largely involved evaluation at the transcriptional level, so the present experiment was designed to extend these analyses through an assessment of receptor binding. In this study, control, SGA and LGA offspring were generated from dams fed control, low protein or high fat diet, respectively, throughout pregnancy and lactation. At weaning, mice were placed on the control diet and sacrificed at 12 weeks of age. *In vitro* autoradiography was used to measure mu-opioid receptor (MOR), dopamine type 1 receptor (D1R), and dopamine transporter (DAT) binding level in mesolimbic brain regions. Results showed that the LP offspring

Correspondence to: Panayotis K. Thanos.

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(males and females) had significantly higher MOR and D1R binding than the control animals in the regions associated with reward. In HF offspring there were no differences in MOR binding, and limited increases in D1R binding, seen only in females in the nucleus accumbens core and the dorsomedial caudate/putamen. DAT binding revealed no differences in either models. In conclusion, LP but not HF offspring show significantly elevated MOR and D1R binding in the brain thus affecting DA and opioid signaling. These findings advance the current understanding of how suboptimal gestational diets can adversely impact neurodevelopment and increase the risk for disorders such as ADHD, obesity and addiction.

Keywords

opioid; dopamine; maternal high fat diet; maternal low protein diet; reward

1. Introduction

In utero insults can increase vulnerability for the development of cardiovascular, metabolic and neuroendocrine disorders in later life (Barker and Osmond, 1986, Barker, 1998). Small for gestational age (SGA) is defined as a birth weight lower than the 10th percentile for fetal growth. The causes for these developmental phenomena are numerous and common. Risk factors include higher order births, maternal malnutrition, smoking or drug use, and, conditions that can lead to a decrease in nutrient flow to the fetus, including maternal hypertension, hyperemesis gravidum, and uterine or placental dysfunction (Cnattingius et al., 1984). It is estimated that 32.4 million neonates were born with SGA in 138 countries of low and middle income, and account approximately for 27% of live births (Lee et al., 2013). The Dutch Hunger Winter and the 1959-1961 Chinese famine provide critical evidence that prenatal malnutrition can lead to an increased risk for neurodevelopmental disorders such as schizophrenia by nearly two fold in adults (Xu et al., 2009, Bale et al., 2010).

On the opposite end of the spectrum are babies born large for gestational age (LGA). LGA is defined as infants that are born with higher than 90th percentile for gestational age. Clinically, two conditions significantly increase the risk for an infant to be born LGA; excessive maternal weight gain during pregnancy (which affects between 50-75% of all pregnancies (Brawarsky et al., 2005, Oken et al., 2007) and maternal obesity (which affects 20-38% of all pregnancies (Yogev and Catalano, 2009). The increasing trend of obesity suggests a parallel increase in the number of obese women during pregnancy (Kanagalingam et al., 2005, Drake and Liu, 2009), indicating a persistent health issue. Epidemiological studies suggest that both LGA and SGA babies have an increased risk for the eventual development of metabolic syndrome, obesity, and mental disorders such as Attention Deficit Hyper Activity Disorder (ADHD), anxiety and depression (Colman et al., 2012, Moore et al., 2012, Grissom and Reyes, 2013). These adverse outcomes on infant brain development further highlight the importance of examining how maternal diets can affect the development of the offspring brain.

The mesocorticolimbic dopaminergic pathway has been shown to play a major role in food reward and obesity (Vucetic and Reyes, 2010, Vucetic et al., 2011, Grissom and Reyes,

2013). In addition, opioid signaling plays an important role in reward signaling pathways, such as the control of food intake (Kelley et al., 2005). Mu opioid receptor (MOR) activation in the striatum enhances high fat diet intake (Zhang and Kelley, 2000), while MOR activation in the cortex enhances carbohydrate intake (Mena et al., 2011). And these two systems interact extensively, as the consumption of high calorie food leads to an increased concentration of endogenous opioids (Zhang et al., 1998), which stimulates dopamine (DA) release by binding to opioid receptors in the ventral tegmental area (VTA) (Bergevin et al., 2002).

Findings from our dietary mouse models reveal changes in DA and opioid related gene expression as well as differences in DA and opioid-dependent behaviors in both LP and HF offspring (Grissom and Reyes, 2013, Grissom et al., 2015b). Significant increases in DA-related gene expression within the reward circuitry are found in the LP offspring (e.g., increased tyrosine hydroxylase and dopamine transporter (DAT) mRNA (Vucetic et al., 2010b)), while opioid related gene expression is downregulated (Grissom and Reyes, 2013). In HF offspring, DAT mRNA was again found to be increased, however expression of D1 and D2 receptor mRNA was decreased. Further, both MOR and PENK were increased in the HF offspring (Vucetic et al., 2010a). An independent group studying maternal high fat diet intake showed higher DAT activity in accumbens (Acb) and attenuated DA type 2 receptors (D2R) in the VTA at the level of mRNA (Naef et al., 2011). These findings suggest that specific adverse experiences during gestation and lactation can drive dysfunction in the DA and opioid systems at the transcriptional level within mesolimbic reward pathway, but do not establish whether these transcriptional changes are reflected in receptor protein expression. Therefore, this experiment sought to demonstrate the effect of maternal diets on protein levels involved in opioid and DA signaling within the mesolimbic system through the use of using in vitro autoradiography. LGA and SGA mouse models were generated to determine whether maternal diet during gestation and lactation (environmental effect) can exert effects on the offspring's receptor level of MORs, DA type 1 receptor (D1R), and DAT using *in vitro* autoradiography.

2. Materials and Methods

2.1 Animals and Diet

Female C57BL/6J and male DBA/2J mice were bred as previously described in Grissom et al. (2013), Grissom et al. (2015a). Three diets were used throughout pregnancy and lactation: Standard Chow diet (SC) (a total energy content of 4.7 kcal/g with 19.8% of total energy calories from protein, 45.9% from carbohydrates, and 25.3% from fat); High fat diet (HF) (a total energy content of 5.1 kcal/g with 18.4% of total energy from protein, 21.3% from carbohydrates, and 60.3% from fat); and low protein diet (LP) (a total energy content of 4.13 kcal/g with 8.5% of total energy calories from protein, 69.5% from carbohydrates, and 22% from fat). Diets were obtained from Test Diet (Richmond, IN, USA). Male (n=5/diet) and female (n=5/diet) C57BL6 X DBA/2J F1 offspring were weaned onto standard chow and sacrificed at 12 weeks of age, and only one animal per sex was used from any one litter, to control for potential litter effects. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2 *In vitro* Quantitative Receptor Autoradiography (ARG)

After harvesting, the brains were flash-frozen, and then stored in -80°C freezer. The brains were later sectioned at 14 micrometers, -20°C , and mounted onto glass slides for quantitative binding. Sections were stored at -80°C until incubation with the ligand of choice and shown below:

MOR Binding—ARG was performed to assess MOR binding levels. Slides were pre-incubated for 30 minutes at room temperature in 50mM Tris HCl buffer with 0.9% NaCl (pH = 7.4). Slides were incubated for 60 minutes at room temperature in pre-incubation buffer in the presence of 5 nM [^3H] [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO), a highly selective MOR agonist (TOCRIS, Missouri, USA). Non-specific binding was determined in the presence of 10 μM naloxone. Slides were washed 2×5 minutes at 4°C in pre-incubation buffer followed by a dip at 4°C in dH₂O. Protocol adapted from Kitchen et al. (1997).

D1R Binding—ARG was performed to assess D1R binding levels. D1R binding was performed with a protocol adapted from Tarazi et al. (1998). Briefly, slides were pre-incubated for 60 minutes at room temperature in 50mM Tris HCl buffer (120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, pH = 7.4). Slides were incubated for 60 minutes at room temperature in pre-incubation buffer in the presence of 0.5 nM [^3H] SCH 23390 (TOCRIS) and 40 nM ketanserin. Non-specific binding was determined in the presence of 10 μM sulpride. Slides were washed 2×5 minutes at 4°C in pre-incubation buffer followed by a dip at 4°C in dH₂O.

DAT Binding—ARG was performed to assess DAT binding levels. Slides were preincubated for 10 minutes at 4°C in 30mM sodium phosphate buffer at pH 7.4. They were incubated for 90 minutes at 4°C in 30mM sodium phosphate buffer solution with 0.32 M sucrose (pH=7.4) and in the presence of 4 nM [^3H] WIN 35,428 (TOCRIS). Non-specific binding was determined in the presence of 30 μM cocaine. Slides were washed 2×5 minutes at 4°C in pre-incubation buffer followed by a dip at 4°C in dH₂O (Hebert et al., 1999).

2.3 Image Analysis

After binding, MOR sections were exposed onto Kodax BioMax MR Film to be developed. All regions of interest (ROIs) were quantified using calibrated standard curves and the NIH Image J software (version 1.47, <http://rsbweb.nih.gov/ij/>). ROIs for MOR were depicted in Figure 1. D1R sections were exposed to Beta Imager (BioSpace, Inc., Paris, France) and the D1R binding was quantified. Beta-vision Plus (BioSpace, France) software was used to determine levels of D1R binding in the ROIs shown in Figure 2. DAT sections were exposed onto Kodax BioMax MR Film. All ROIs were quantified as described above using calibrated standard curves and the NIH Image J software. ROIs were shown in Figure 3. Brain regions were analyzed by manually ROIs on each specimen and comparing densities across groups. The average density was taken per group to compare levels of ligand binding indicating the concentration of receptors. Specific binding was determined by subtracting non-specific from total binding.

2.4 Statistics

For MOR, D1R and DAT binding levels, a 2-way ANOVA was performed for each region of interest with factors sex and treatment. Pairwise comparisons were performed using the Holm-Sidak method. All statistical tests were performed using Sigmaplot 11.0 software (Systat Software, Inc., Chicago, Illinois). $P < 0.05$ was considered statistically significant. The comparisons of interest were SC vs LP and SC versus HF, and to minimize animal duplication, only a single control group was used.

3. Results

In vitro autoradiography of [^3H]DAMGO binding to MORs, [^3H] SCH 23390 binding to D1Rs, and [^3H]WIN 35,428 binding to DATs was assessed throughout ROIs in SGA and LGA mice. The representative images are shown in Figures 1-3.

The signal in [^3H]DAMGO-bound autoradiographs was broadly distributed, and high levels of binding were noticed in the four quadrants of caudate putamen (CPu), habenula (hb), and ventral tegmental area (VTA) consistent with a previous report using [^3H]DAMGO (Kitchen et al., 1997) (Figure 1A). Autoradiographic image analysis revealed no differences in MOR binding in the HF offspring brains (Figure 1B). LP offspring, however demonstrated significant increases in MOR binding across numerous brain regions (Figure 1C). Significant main effects of maternal diet were observed in several regions, including olfactory tubercle [$F(1,16) = 6.098$, $p < 0.05$], nucleus accumbens shell (AcbSh) [$F(1,16) = 4.906$, $P < 0.05$], Acb core (AcbC) [$F(1,16) = 7.621$, $P < 0.05$], ventral medial (VM) CPu [$F(1,16) = 11.97$, $P < 0.01$], ventral lateral (VL) CPu [$F(1,16) = 5.174$, $P < 0.05$], Hb [$F(1,16) = 4.816$, $P < 0.05$], amygdala (amg) [$F(1,16) = 5.132$, $P < 0.05$], hypothalamus (HYP) [$F(1,16) = 4.711$, $P < 0.05$], VTA [$F(1,16) = 11.966$, $P < 0.01$], and superior colliculus (SCo) [$F(1,16) = 6.223$, $P < 0.05$]. Neither the main effects of sex, nor the treatment $\times$ sex interactions were significant for any brain region analyzed. Pairwise multiple comparisons using Holm-Sidak method revealed that LP groups exhibited greater MOR binding than SC groups in the Tu (31.2%, $P < 0.05$), AcbSh (18.4%, $P < 0.05$), AcbC (22.3%, $P < 0.05$), VM CPu (35.2%, $P < 0.01$), and VL CPu (22.4%, $P < 0.05$), Hb (29.5%, $P < 0.05$), amg (23.5%, $P < 0.05$), Hyp (22.7%, $P < 0.05$), VTA (44.3%, $P < 0.01$), and SCo (33.8%, $P < 0.05$). Further, females demonstrated significant increases in binding in the AcbC (32.7%, $P < 0.05$), AcbSh (30.1%, $P < 0.05$), VM CPu (35.2%, $P < 0.01$), and VTA (55.0%, $P < 0.01$), suggesting that in these regions, female responses were likely driving more of the observed main effect.

In both D1R and DAT bindings, very high levels of binding were shown in the CPu, nucleus accumbens core and shell (AcbC and AcbSh), and olfactory tubercle (Fig 2A, 3A). Similar to MOR binding results, for D1R binding, very few differences were observed in the HF offspring, aside from two brain regions (Fig 2B). Within AcbC and DM CPu, female HF offspring displayed an increase in D1R binding, with HF groups exhibiting greater D1R binding than SC group in the AcbC (43.1%, $p < 0.05$), and DM CPu (36.7%, $p < 0.05$).

However, similar to the MOR findings, significant differences were observed in the LP offspring. Autoradiographic analysis of D1Rs showed that there was a significant main effect of treatment in the Tu [$F(1,16) = 11.746$, $p < 0.01$], AcbSh [$F(1,16) = 5.882$, $p < 0.05$],

AcbC [$F(1,16)=11.22$, $p<0.01$], DM CPu [$F(1,16)=8.461$, $p=0.01$], VM CPu [$F(1,16)=8.008$, $p<0.05$], and VL CPu [$F(1,16)=8.914$, $p<0.01$]. DL CPu [$F(1,16)=4.497$, $p=0.051$] (Fig 2C). Neither the main effects of sex, nor the treatment $\times$ sex interactions were significant for any brain region analyzed. Pairwise multiple comparisons using Holm-Sidak method revealed that LP groups exhibited greater D1R binding than SC groups in the Tu (23.0%, $p<0.01$), AcbSh (31.7%, $p<0.05$), AcbC (45.6%, $p<0.01$), DM CPu (42.1%, $p=0.01$), VM CPu (39.3%, $p<0.05$), and VL CPu (43.0%, $p<0.01$). Within females, LP groups displayed greater D1R binding than SC groups in the Tu (32.0%, $p<0.05$), AcbSh (44.9%, $p<0.05$), AcbC (65.4%, $p<0.01$), DM CPu (57.2%, $p<0.05$), VM CPu (59.4%, $p<0.05$), VL CPu (61.7%, $p<0.01$), again suggesting that within these regions, female responses were likely driving more of the observed main effect.

For DAT binding, none of the brain ROIs showed any treatment, or sex significance compared to SC mice (Fig 3B, 3C).

24. Discussion

The main objective of this study was to address the effect of prenatal exposure to suboptimal maternal diets, on opioid and DA receptor expression within central reward pathway. The data are congruent with many other studies showing that insults during pregnancy and lactation can persist into adulthood (Vucetic et al., 2010a), (Vucetic et al., 2010b). The rationale for this study was our previous findings that have reported changes in MOR, D1R and DAT mRNA expression in both LP and HF offspring, as well as behavioral differences in reward related behaviors. (Carlin et al., 2013, Grissom et al., 2013) and (Vucetic et al., 2010a), (Vucetic et al., 2010b). The present study significantly extends our understanding of changes within the brains of LP and HF offspring, with a direct examination of ligand binding to expressed receptors. To our knowledge, this experiment is the first to show the effect of maternal consumption of HF and LP diets on offspring's MOR, D1R and DAT binding level and its distribution in the CNS using *in vitro* quantitative autoradiography, with a focus on the reward circuits of the brain.

In the current study, the density of MOR binding is significantly higher in LP offspring than in controls, while HF offspring did not differ from controls. The elevated binding is observed in areas associated with mesolimbic reward pathway, as well as amygdala, habenula, hypothalamus, and superior colliculus. It is currently unclear whether MOR over-expression is accounted for by an increase in cell numbers (cell proliferation) or in receptor peptides per cell (MOR protein upregulation). However, in the hypothalamus, a greater relative volume of the ventromedial hypothalamic nucleus and a greater neuron density in that area have been reported in mice exposed to maternal LP malnutrition (Plagemann et al., 2000), supporting increased neurogenesis as a potential underlying mechanism. The pattern of MOR binding is not congruent with our previous findings that showed significant increases of MOR mRNA expression in LGA offspring (Vucetic and Reyes, 2010) and decreases in LP offspring (Grissom and Reyes, 2013). That suggests that new MOR synthesis is not required for the increased binding observed in the present study. Further, it is important to note that mRNA production does not necessarily equate to the translational and expression of the protein (Pan et al., 2005). Many studies have failed to find a correlation

between mRNA level and protein abundance in different species (Gygi et al., 1999, Chen, 2002, Maier et al., 2009, Taniguchi et al., 2010). Several factors may explain why MOR binding patterns are not consistent with prior results on MOR mRNA (Peckys and Landwehrmeyer, 1999). Low MOR binding observed in this region with autoradiography may be a consequence of a favored binding of [³H] DAMGO to high affinity states of the MOR. This assay may, therefore, result in an underestimation of MOR binding sites in the regions associated with mesolimbic pathway (Peckys and Landwehrmeyer, 1999). There are at least 27 alternatively splice variants of mouse MOR gene that have been isolated and a single mammal MOR gene (OPRM1) has been identified (Pan et al., 2005). It remains unclear how the OPRM1 gene can single-handedly explain the complexity of mu opioids in rodent models (Pan et al., 2005, Doyle et al., 2007, Xu et al., 2011). Collectively, the data suggest that perinatal programming by maternal LP diets in particular brain areas may influence the turnover rate of MOR, leading to a changes at the level of both mRNA and protein expression, albeit in opposite directions. MOR turnover rate may also be profoundly impacted by differences in MOR receptor subtypes and splice variants.

Perturbations of the DA system are closely related with drug addiction, obesity, in addition to many cognitive diseases (Wang et al., 2004, Thanos et al., 2007, Trantham-Davidson et al., 2014). Maternal diets appear to contribute to the formations of mood disorders and impulse control disorders, in addition to dysfunction of the DA system in offspring (Grissom and Reyes, 2013) (Grissom et al., 2015b). Our models of SGA and LGA show disruptions in protein levels of the DA signaling, primarily observed in D1R of the LP offspring. We demonstrate here an increase in D1R binding broadly throughout the reward circuitry of LP offspring. These findings are consistent with our past behavioral report of these animals in which we showed that male SGA animals had a significantly increased locomotor response and enhanced neuronal activation in the NAc in response to cocaine (Vucetic et al., 2010b). Interestingly, the increased D1R binding in SGA animals was greater in magnitude in females in the nucleus accumbens and partial striatum, suggesting a greater responsiveness of the female brain to dietary changes. For the HF offspring, a more limited increase in D1R binding was observed, restricted to AcbC and partial caudate. In rats, another autoradiographic study of D1R binding found no change in the nucleus accumbens and striatum in male rats (Naef et al., 2008), although significant methodological differences between these two studies (species, diet, and duration of exposure) limit the ability for direct comparisons. And lastly, in order to more fully understand potential effects on DA transmission, DAT must be considered. The primary mechanism of DA clearance in the extrasynaptic space is executed by DAT through reuptake. In the present experiments, no differences in DAT binding were observed in either LP or HF offspring. However, our own behavioral data demonstrate an exaggerated locomotor response to cocaine in both LP and HF offspring (Vucetic and Reyes, 2010), (Grissom and Reyes, 2013), suggesting that indeed the DA system is altered. Future studies incorporating more functional assays will be required to fully dissect that ways in which early life adversity changes dopaminergic function within mesolimbic circuitry. This study provides further support for the hypothesis that prenatal exposure to suboptimal nutrition has lasting effects on DA and opioid systems within the central reward pathway. In regard to receptor density, both DA and opioid systems were affected by maternal low protein diet during pregnancy and lactation, but only the DA

system was impacted in offspring from maternal high fat dams, and to a lesser extent than that seen in the LP animals. Further, the observed effects on receptor binding were notably stronger in females, suggesting an important sex difference in the response of the DA and opioid systems to prenatal malnutrition. There are well known sex biases in the prevalence of many neurodevelopmental disorders (Davis and Pfaff, 2014), and differential responses of D1R and/or MOR binding could potentially contribute to those observed sex differences. In conclusion, the effect of maternal diets suggests that these receptors may be linked to the mechanism responsible for the increased health risks such as: developing metabolic syndrome, obesity, and mental disorders such as ADHD, schizophrenia and depression.

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Highlights

- Maternal low protein diet increases Mu Opioid and D1R binding in offspring
- Maternal high fat diet offspring showed increased D1R binding
- Suboptimal gestational diets adversely impact risk for neurodevelopmental disease

Figure 1. Qualitative and quantitative autoradiographic binding of MOR in HF and LP offspring. Representative images show binding of [^3H] DAMGO in the offspring fed from SC (a), HF (b), and LP (c) diet dams. Schematic delineations of [^3H] DAMGO binding to MORs from rostral to caudal positions in coronal brain sections showing ROIs, including frontal-parietal cortex (frp), caudate putamen (cp) (dorsal medial (DM), dorsal lateral, ventral medial (VM), and ventral lateral (VL)), nucleus accumbens core (AcbC), Acb shell (AcbSh), endopiriform nucleus(en), olfactory tubercle (Tu), hippocampus (hpc), habenula (hb), thalamus (thl),

amygdala (amg), hypothalamus (hyp), superior colliculus (SCo), medial geniculate (mg), substantia nigra (SNr), red nucleus (rn), ventral tegmental area (VTA), and interpeduncular complex (ip) (d). [³H] DAMGO standards ranging from 0 to 18.4 uCi/g were counted (e). Freehand drawings are according to the mouse atlas of Franklin and Paxinos (2007). In HF diet fed mice, none of the brain ROIs show treatment, or sex significance, but increasing trend of MOR binding is noticed in female HF diet compared to SC mice (f). In offspring fed from LP diet, significant treatment difference was shown compared to SC mice, and within females, significant treatment difference was also displayed (g). Values are mean +SEM of five animals per diet group. * $p < 0.05$, LPSC > SCSC; ** $p < 0.01$, LPSC > SCSC; $^{\$}p < 0.05$, LPSC Female > SCSC Female; $^{\$\$}p < 0.01$, LPSC Female > SCSC Female.

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Figure 2.

Qualitative and quantitative autoradiographic binding of D1R in in HF and LP offspring. Representative images show binding of [³H] SCH 23390 in the offspring fed from SC (a), HF (b), and LP (c) diet dams. Schematic delineations of [³H] SCH 23390 binding to D1Rs from rostral to caudal positions in coronal brain sections showing ROIs, including Tu, Acb, AcbSh, AcbC, DM CPu, DL CPu, VM CPu, VL CPu, DL CPu Tail, VL CPu Tail, VTA and SNr (d). [³H] SCH 23390 standards ranging from 0 to 0.4871 uCi/g were counted (e). Freehand drawings are according to the mouse atlas of Franklin and Paxinos (2007). In HF

diet fed mice, only AcbC and DM CPu show treatment significance within females compared to SC mice (f). In offspring fed from LP diet, significant treatment difference was shown compared to SC mice, and within females, significant treatment difference was also displayed (g). Values are mean+SEM of five animals per diet group. ¹p<0.05, HFSC Female > SCSC Female; *p<0.05, LPSC>SCSC; **p<0.01, LPSC>SCSC; \$p<0.05, LPSC Female> SCSC Female; \$\$p<0.01, LPSC Female> SCSC Female.

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Figure 3.

Qualitative and quantitative autoradiographic binding of DAT in HF and LP offspring. Representative images show binding of [^3H] WIN 35,428 in the offspring fed from SC (a), HF (b), and LP (c) diet dams. Schematic delineations of [^3H] WIN 35,428 binding to DAT from rostral to caudal positions in coronal brain sections showing ROIs, including Tu, AcbSh, AcbC, DM Cpu, DL Cpu, VM Cpu, VL Cpu, DL Cpu Tail, VL Cpu Tail, VTA and SNr (d). [^3H] WIN 35,428 standards ranging from 0 to 18.4 uCi/g were counted (e). Freehand drawings are according to the mouse atlas of Franklin and Paxinos (2007). In both

HF and LP diet fed mice, none of the brain ROIs show treatment, or sex significance compared to SC mice (f) (g).

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