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EXPRESSION OF DOPAMINE SIGNALING GENES IN THE POST-MORTEM BRAIN OF INDIVIDUALS WITH MENTAL ILLNESSES IS MODERATED BY BODY MASS INDEX AND MEDIATED BY INSULIN SIGNALING GENES

Rodrigo B. Mansur, MD, PhD^{1,2,*}, Gabriel R. Fries, PhD³, Mehala Subramaniapillai, MSc¹, Sophia Frangou, MD, PhD⁴, Fernanda G. De Felice, PhD⁵, Natalie Rasgon, MD, PhD⁶, Bruce McEwen, PhD⁷, Elisa Brietzke, MD, PhD^{1,8}, and Roger S. McIntyre, MD^{1,2}

¹Mood Disorders Psychopharmacology Unit (MDPU), University Health Network, University of Toronto, Toronto, Canada

²University of Toronto, Toronto, Canada

³Department of Psychiatry and Behavioral Sciences, McGovern Medical School at the University of Texas Health Science Center at Houston, Houston, USA.

⁴Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, USA.

⁵Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; Centre for Neuroscience Studies, Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada.

⁶Center for Neuroscience in Women's Health, Stanford University, Palo Alto, USA.

⁷Laboratory of Neuroendocrinology, The Rockefeller University, New York, USA

⁸Department of Psychiatry, Universidade Federal de São Paulo, São Paulo, Brazil; Research Group in Molecular and Behavioral Neuroscience of Bipolar Disorder, Department of Psychiatry, Universidade Federal de São Paulo, SP, Brazil.

Abstract

Preclinical studies implicate insulin signaling as a modulator of dopamine transmission, but human data is currently limited. We hypothesize that changes in the expression of insulin receptor-related genes in the post-mortem brain tissue of patients with mood and psychotic disorders mediate the expression of dopamine regulation-related genes. From a database containing microarray data from the post-mortem dorsolateral prefrontal cortex (dlPFC) (healthy controls [HC]: n = 209; patients: n = 321) and hippocampus (HC: n = 180; patients: n = 196), we conducted a hypothesis-driven analysis through the a priori selection of 12 dopamine- and 3

*Corresponding author: Rodrigo B. Mansur, 399 Bathurst Street, MP 9-325, Toronto, Ontario, M5T 2S8, Canada, Phone: (416) 603-5800, Fax: (416) 603-5368, rodrigo.mansur@uhn.ca.

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insulin-related genes. Mediation and moderated mediation models, accounting for the role of body mass index (BMI), were used. In the dlPFC, expressions of insulin receptor- and dopamine regulation-related genes were moderated by BMI, with significantly lower expression in high BMI patients. In the hippocampus, there were significantly lower expressions of these genes, which were not moderated by BMI. Illnesses by BMI effects on expression of dopamine genes were fully mediated by expression of insulin receptor gene (INSR). Analysis of conditional indirect effects showed interactions between INSR and BMI, indicating significantly stronger indirect effects at higher BMI values. In the hippocampus we observed that expression of insulin receptor substrate 1 and 2 fully mediated the effects of illnesses on expression of dopamine genes. In conclusion, differential expression of dopamine-related genes was related to altered expression of insulin signaling genes. BMI had region-specific effects, supporting the hypothesis that metabolic systems are critical mediators of dopaminergic function.

Keywords

Dopamine; Mood Disorders; Schizophrenia; Gene Expression; Insulin

1. Introduction

Abnormalities in dopaminergic neurotransmission are critically involved in the pathophysiology of psychotic and mood disorders. Dopamine abnormalities have been associated with positive psychotic symptoms (Howes and Kapur, 2009) and with mood dysregulation related to mania and anhedonic depression (Grace, 2016). Dopamine release in the mesolimbic pathway, which consists of dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (Russo and Nestler, 2013), has been associated with modulation of mood, attention, cognition and reward (Beaulieu and Gainetdinov, 2011). These areas are heavily interconnected with the prefrontal cortex (PFC), amygdala and hippocampus, among other areas (Russo and Nestler, 2013). Specifically, connections between the dorsolateral prefrontal cortex (dlPFC) and the NAc and VTA, have a key role in motivated behavior (Ballard et al., 2011). Converging gene expression, imaging and pharmacological studies in individuals with psychotic and mood disorders have documented abnormalities in dopaminergic neurotransmission (Ashok et al., 2017, Purves-Tyson et al., 2017). For example, imaging studies have demonstrated alterations in dopamine receptor and transporter binding in individuals with psychotic and mood disorders (Anand et al., 2011, Camardese et al., 2014, Cannon et al., 2009, Hirvonen et al., 2005, Meyer-Lindenberg et al., 2002).

Dopamine signaling within and across brain circuits is in interplay with other systems, including the insulin signaling pathway (Kleinridders et al., 2015, Stouffer et al., 2015). Insulin acts via the insulin receptor (INSR). Activation of INSR initiates the recruitment of the insulin receptor substrate (IRS) family of proteins, which organize and mediate signaling cascades (White, 2006). IRS1 and IRS2 are the most extensively studied substrates; they are expressed in the brain and have well-documented effects on insulin signaling and glucose homeostasis (Lin et al., 2004, Pardini et al., 2006). Animal studies have documented that insulin receptors are widely expressed in midbrain dopamine neurons (Figlewicz et al.,

2003). Transdisciplinary preclinical/clinical research indicates that insulin signaling influences dopamine availability and turnover (Kleinridders, Cai, 2015, Stouffer, Woods, 2015). Synthesis is regulated by tyrosine hydroxylase (TH), which converts L-tyrosine to L-DOPA, which is then converted by dopamine decarboxylase (DDC) to dopamine. Dopamine is transported to synapses by the vesicular monoamine transporter 2 (VMAT2). In animal models of diabetes, lower expression and function of TH have been reported (Bradberry et al., 1989, Kono and Takada, 1994); conversely, administration of insulin has been shown to induce dopamine release in the NAc and the striatum of rats (Potter et al., 1999).

Upon release, dopamine binds to dopamine receptors (DR), conventionally grouped into the inhibitory D2-like family (DRD2, DRD3, DRD4) and the excitatory D1-like family (DRD1 and DRD5). In humans, insulin resistance was associated with lower endogenous dopamine at D2/3 receptors in the ventral striatum and NAc, in both obese and non-obese participants (Caravaggio et al., 2015a, Dunn et al., 2012). A separate study documented that weight loss following bariatric surgery was associated with an increase in D2 receptor availability (Steele et al., 2010). Termination of dopamine action is via reuptake from the synaptic cleft by the dopamine transporter (DAT), or by degradation by monoamine oxidases (MAOs) A/B and catechol-O-methyltransferase (COMT). Studies with animal models of diabetes have reported lower availability and activity of DAT (Figlewicz et al., 1996, Petrisic et al., 1997) and lower activity of COMT (Wang et al., 2002); whereas a murine model with a brain specific knockout of the insulin receptor (i.e. NIRKO model) found that decreased insulin function in the brain resulted in increased expression of MAO A/B in the striatum and NAc (Kleinridders, Cai, 2015).

Replicated lines of inquiry indicate that mental and metabolic disorders have a bidirectional association (Luppino et al., 2010, Pan et al., 2012). Epidemiological studies have documented a high prevalence of comorbid metabolic disorders in individuals with mental illness (MI) (i.e. schizophrenia [SCZ], major depressive disorder [MDD] and bipolar disorder [BD]). For example, meta-analytic studies have documented that individuals with mood disorders have an approximately 2-fold increased risk of type 2 diabetes mellitus (T2DM), relative to the general population (Vancampfort et al., 2015a). It is estimated that the prevalence of T2DM in individuals with mood disorders is between 8 and 10% (Vancampfort, Mitchell, 2015a), whereas the prevalence of metabolic syndrome is approximately 30% (Vancampfort et al., 2015b). Conversely, it is reported in an adult population that individuals with T2DM have an approximate 2–3 fold greater risk for subsequently declaring MDD or BD (Wahlqvist et al., 2012). Clinical studies have reported an onset of metabolic comorbidities early, or even prior to the declaration of a MI (Pillinger et al., 2017), and a higher risk of metabolic disorder was also reported in unaffected first-degree relatives of patients with psychotic disorders (Mothi et al., 2015). A recent genetic study identified multiple genes that are likely shared between mood and metabolic disorders; pathway analysis revealed an over-representation of genes that encode for molecules involved in dopamine signaling (Amare et al., 2017). Indeed, anhedonia has been consistently reported in T2DM populations, independently of other depression measures, and has been associated with an increase incidence of complications and all-cause mortality (Nefs et al., 2016). The foregoing collection of observation provide the rationale to

hypothesize that alterations in insulin signalling are associated with and/or mediate alterations in dopamine signalling in individuals with MI.

The primary aim of this study was to investigate the relationship between the expression of dopamine and insulin-related genes, using data from a post-mortem study, which consists of samples from the hippocampus and dorsolateral prefrontal cortex (dlPFC) of a population of healthy controls [HC] and individuals with SCZ, MDD or BD. The primary hypothesis was that a differential expression of insulin signaling genes, between HCs and individuals with MI, would mediate the differential expression of dopamine signaling genes. Specifically, we hypothesized that expression of insulin signaling genes (i.e. INSR, IRS1 and IRS2) would be associated with expression of genes involved in dopamine synthesis and transport (i.e. TH, DDC and VMAT2), receptor function (i.e. DRD1, DRD2, DRD3, DRD4, DRD5) and reuptake/degradation (i.e. COMT, DAT, MAOA and MAOB); and that differences in the expression of insulin receptor-related genes in patients, relative to controls, would be related to changes in the expression of dopamine regulation-related genes. As obesity has been shown to modulate gene expression in the brain (Lauridsen et al., 2017), the secondary hypothesis of this study is that obesity would moderate gene expression and any mediation effects of insulin signaling genes.

2. Material and Methods

2.1 Sample selection

The “Gene Expression in Postmortem DLPFC and Hippocampus from Schizophrenia and Mood Disorders” (dbGaP accession number phs000979.v1.p1) database contains microarray data from the hippocampus and dlPFC across nine major mental disorders, with emphasis on SCZ, BD and MDD. Postmortem brains were collected at the Human Brain Collection Core, NIMH with informed consent from the legal next of kin (NIMH protocol 90-M-0142), and at the Brain and Tissue Bank for Developmental Disorders of the NICHD (contracts NO1-HD-4-3368 and NO1-HD-4-3383) and through the Stanley Medical Research Institute. The clinical characterization, neuropathological screening, toxicological analyses, and dissection of the dlPFC and hippocampus have been previously described (Lipska et al., 2006). Lifetime Axis I diagnosis were based on DSM-IV criteria; controls had no history of psychological or psychiatric problems and negative toxicology results.

The database includes 914 subjects from whom 849 specimens from the dlPFC and 579 from the hippocampus were available. We excluded individuals with diagnoses other than SCZ, MDD or BD (dlPFC = 53, hippocampus = 25), individuals younger than 18 (dlPFC = 111, hippocampus = 83) or older than 65 years of age (dlPFC = 56, hippocampus = 49). As there were only 46 subjects of Hispanic or Asian ethnicity we chose also to exclude them. We further excluded 28 dlPFC and 9 hippocampal samples due to post-mortem interval (PMI) > 72hs. Forty-four subjects from the dlPFC and 17 from the hippocampus sample were excluded due to lack of laboratorial measures. All samples analyzed had RNA integrity numbers (RINs) above 5.

The final dataset comprised 530 specimens from the dlPFC, of which 321 were from individuals with MI (SCZ = 142, MDD = 99, BD = 80); and 376 hippocampal samples, of

which 196 were from patients (SCZ = 102, MDD = 52, BD = 42). A subset of subjects also had BMI data available. In the dlPFC sample, 413 subjects had BMI data, of those 15 subjects with outlying values (1 due to BMI below 12 and 14 due to BMI above 50) were excluded. In the hippocampus data, 365 subjects had BMI data available; of those we excluded 14 subjects with outlying values (1 due to BMI below 12 and 13 due to BMI above 50). All the data were anonymized in accordance with the rules and regulations of the National Institute of Health.

2.2 mRNA analyses

We analysed mRNA data obtained using HumanHT-12 v4 BeadChip arrays (Illumina). The genes INSR, IRS1, IRS2, DDC, TH, VMAT2, DRD1, DRD2, DRD3, DRD4, DRD5, COMT, DAT, MAOA and MAOB were selected *a priori* for analysis, based on their previously reported involvement in dopamine and insulin signaling. Gene symbols, names and probes are described in Supplementary Table S1. Analysis of these selected genes by the Ingenuity Pathway Analysis (IPA®, Qiagen) confirmed their involvement in dopamine receptor signaling (top-ranked canonical pathway; $p = 3.75 \times 10^{-27}$) and in a complex network involving several other genes (Supplementary Figure S1). For genes with more than one probe, the average from all probes was used. Gene expression mean values were z-transformed (Cheadle et al., 2003). Outliers values, defined as more than 5 standard deviations (SD) above the mean, were excluded.

2.3 Analytic Plan

Raw data obtained from the Illumina Human V4-HT12 arrays were analyzed with the Bioconductor limma package, as previously described (Ritchie et al., 2015). Multivariate analysis of covariance (MANCOVA) were used to investigate the main effects of group (HC vs. MI), as well as of relevant covariates. Interactions were assessed by adding the product term (i.e. group*BMI) to separate models. Due to deviation of normality, a bootstrapping method, with 1,000 simple bootstrap samples with bias corrected and accelerated (BCa) 95% confidence interval, was used.

To assess mediation and moderation, we used the PROCESS macro (Hayes, 2013) in SPSS v24.0 (IBM Corp., 2013). The main objective was to test whether there is a mediated effect of X (MI) on Y (expression of dopamine signaling genes) through M (expression of insulin signaling genes). If appropriate, moderated mediation models (conditional process analysis) were used to test whether the mediation effect was contingent on W (BMI) (Supplementary Figure S2). The models were run separately for each selected outcome (i.e. dopamine-related gene).

Bootstrapping with 5,000 resamples was performed to determine bias-corrected (asymmetric) 95% confidence intervals for indirect effects (Preacher and Hayes, 2008). We opted not to correct for multiple comparisons as this was a hypothesis-driven analysis and the outcomes (i.e. dopamine signaling genes) were highly correlated.

3. Results

3.1 Mental illness and gene expression involved in the dopamine and insulin signaling pathways

Table 1 describes the sample characteristics. There were differences in gender and race compositions of the samples, with a higher frequency of females and Caucasian in the patients group. Healthy controls also had a slightly higher mean BMI. Patient's samples had significantly higher frequency of positive laboratorial tests for ethanol, nicotine/cotinine, benzodiazepines, THC, cocaine, opiates, antipsychotics, antidepressants and mood stabilizers'. First, we assessed the effects of potential confounders using multivariate analyses. Results indicated that the potential confounders that had an effect on gene expression were, in the dlPFC, age at death ($p < 0.001$), gender ($p = 0.007$), race (Caucasian vs. African American) ($p < 0.001$), ethanol ($p = 0.026$) and nicotine/cotinine ($p = 0.008$); and in the hippocampus, only age at death ($p < 0.001$). Gender ($p = 0.264$), race ($p = 0.275$), ethanol ($p = 0.085$) and nicotine/cotinine ($p = 0.522$) did not have an effect on hippocampal gene expression; benzodiazepines, THC, cocaine, opiates and, within patients, antipsychotics, antidepressants and mood stabilizers also had non-significant effects in both the dlPFC and the hippocampus (all $ps > 0.1$).

After adjustment for age, gender, race, ethanol and nicotine/cotinine, there were significant group effects (HC vs. MI) on the dlPFC (Wilks' λ $F_{15,500} = 3.978$, $p < 0.001$, $\eta^2 = 0.107$) and hippocampus (Wilks' λ $F_{15,345} = 2.562$, $p = 0.001$, $\eta^2 = 0.100$). Subsequently, a separate analyses, including further BMI and an interaction term (BMI * MI) was conducted (Table 2). The BMI by illnesses interaction was significant in the dlPFC (Wilks' λ $F_{15,384} = 2.261$, $p = 0.003$, $\eta^2 = 0.081$), but not in the hippocampus (Wilks' λ $F_{15,319} = 0.801$, $p = 0.676$, $\eta^2 = 0.036$). BMI effects were non-significant in both regions ($p = 0.110$ and $p = 0.169$, respectively).

Table 2 shows the between group differences in individuals genes expressions. Expression of DRD4, MAOA and MAOB were increased, whereas of DRD1 was decreased, in the dlPFC of individuals with MI. Expressions of INSR, IRS1, IRS2, DDC, VMAT2, DRD2, DRD5 and DAT were moderated by BMI, with significantly lower expression values in higher BMI patients (Figure 1). In the hippocampus, we observed lower expression of IRS1, IRS2, TH, DRD1 and MAOB, which were not influenced by BMI.

3.2 Mediation analyses

Genes that were differentially expressed between groups were selected for mediation analyses. As gene expression changes in INSR, IRS1 and IRS2 in the dlPFC were moderated by BMI, we used moderated mediation models (conditional process analysis) (Supplementary Figure S2); in the hippocampus samples we used simple mediation models. All models were adjusted for age, gender, race, ethanol and nicotine/cotinine. Results from the dlPFC sample are shown in Table 3. Illnesses by BMI effects on expression of DDC, VMAT2, DRD2, DRD5, COMT and DAT were fully mediated by expression of INSR, insofar as this interaction was only significant in the models that included the mediators. There were no mediating effects of IRS1 or IRS2. There were as well interactions between

INSR and BMI on the outcomes DDC and VMAT2, with analysis of conditional indirect effects indicating significantly stronger indirect effects of the mediators at higher BMI values. All models were significant (DDC: $R^2 = 0.533$, $F_{10,400} = 45.732$, $p < 0.001$; VMAT2: $R^2 = 0.498$, $F_{10,400} = 39.745$, $p < 0.001$; DRD2: $R^2 = 0.392$, $F_{10,400} = 25.752$, $p < 0.001$; DRD5: $R^2 = 0.199$, $F_{10,400} = 9.937$, $p < 0.001$; COMT: $R^2 = 0.119$, $F_{10,400} = 5.402$, $p < 0.001$; and DAT: $R^2 = 0.479$, $F_{10,399} = 36.726$, $p < 0.001$).

In the hippocampus we observed that expression of IRS2 fully mediated the effects of MI on expression of TH; whereas IRS1 and IRS2 fully mediated the effects of MI on and DRD1 and MAOB (Table 4). There were no mediating effects of INSR. All models were also significant (TH: $R^2 = 0.157$, $F_{8,365} = 8.483$, $p < 0.001$; DRD1: $R^2 = 0.337$, $F_{8,366} = 23.264$, $p < 0.001$; MAOB: $R^2 = 0.341$, $F_{8,367} = 23.791$, $p < 0.001$).

4. Discussion

We observed that, in individuals with BD, MDD and SCZ, expression of dopamine regulation-related genes in the dlPFC was moderated by BMI; and that in both the dlPFC and in the hippocampus they were mediated by expression of insulin receptor-related genes. Lower expression of genes involved in the regulation of dopamine neurotransmission (i.e. DDC, TH, VMAT2, DRD1, DRD2, DRD5, and MAOB), indicating lower dopamine metabolism, were fully mediated by a lower expression of insulin receptor-related signaling genes (i.e. INSR, IRS1 and IRS2), which are suggestive (albeit not a direct proxy) of impaired insulin receptor function. These results are largely consistent with the preclinical and clinical data on the association between insulin and dopamine signaling (Caravaggio et al., 2015b, Figlewicz and Sipols, 2010).

Dopamine signaling has been implicated in arousal, motivation and psychomotor activation (i.e. incentive salience models), and reward learning/reinforcement (i.e. prediction error models) (Beierholm et al., 2013, Hamid et al., 2016, Steinberg et al., 2013). Contemporary models posit that dopamine signals value, or the available reward for a certain effort, which is employed for both learning and motivational functions (Hamid, Pettibone, 2016). In addition to insulin receptors, midbrain dopamine neurons express receptors for numerous circulating signals associated with homeostatic mechanisms, including leptin, ghrelin and orexin (Figlewicz and Sipols, 2010). Insulin, as well as other homeostatic inputs, is thought to modulate dopamine signaling and regulate reward processes, particularly those pertaining feeding behaviors and energy expenditure (Beeler et al., 2012, Khanh et al., 2014). Replicated evidence indicates that pharmacological blockage of DRs attenuates feeding and responses to food reward (MacDonald et al., 2004); whereas activation of dopamine neurons has been associated with disruption of feeding patterns (Boekhoudt et al., 2017).

Moreover, it has been proposed that lower dopamine signaling may favor energy conservation and exploitation, resulting in less “willingness to work”, or investing time and effort in activities that are not inherently rewarding or interesting; and may disturb reward expectation and learning (Hamid, Pettibone, 2016, Kroemer and Small, 2016). Indeed, individuals with MI have been repeatedly shown to display a decrease in the willingness to

expend effort and/or in the modulation of effort expenditure for rewards (Clery-Melin et al., 2011, Treadway et al., 2012, Yang et al., 2014). For example, it has been demonstrated that individuals with depression display reduced motivation on effort-based decision making (Clery-Melin, Schmidt, 2011, Treadway, Bossaller, 2012, Yang, Huang, 2014), a dissociation between liking a reward and the willingness to exert effort for it (Sherdell et al., 2012) and a lower ability to effectively use information about rewards to modify their behavioral choices (Treadway, Bossaller, 2012). Conversely, in obesity, whereas the anticipatory reward response seems to be increased (Kroemer and Small, 2016, Verdejo-Roman et al., 2017), the willing to invest effort has as well been shown to be diminished (Mathar et al., 2015).

We observed regional differences regarding the effects of BMI, which was present in the dlPFC, but not in the hippocampus. Indeed, neuroimaging studies have documented divergent regional associations between BMI and brain structure and function (Opel et al., 2017, Weise et al., 2017). There is as well replicated evidence that obesity is associated with abnormal brain dopamine signaling and related gene expression, in non-psychiatric subjects (Volkow et al., 2011, Wu et al., 2017). In our study, gene expression of dopamine and insulin genes was significantly lower in subjects with MI and higher BMI; the mediating effect of expression of INSR on expression of dopamine genes was as well moderated by BMI, suggesting that this maybe a particularly vulnerable subgroup for the deleterious effects of insulin signaling disturbances. This is further supported by reports showing that individuals with a mood disorder and comorbid obesity, insulin resistance or T2DM have an unfavorable clinical course, characterized by increased chronicity, higher risk of suicide, treatment resistance and functional disability (Mansur et al., 2016, Veltman et al., 2017). Several biological pathways have been proposed to explain the association between mood and metabolic disorders, including, but not limited to, mitochondrial, oxidative and inflammatory mechanisms (Mansur et al., 2015). Conversely, these systems could be involved in the documented associations between BMI and expressions on dopamine- and insulin-related genes in the dlPFC. For example, a recent study reported that, in neurologically and psychiatrically healthy humans, higher BMI was associated with decreased expression of a gene involved in anti-inflammatory defense (i.e. interleukin-10), and with upregulation of nitric oxide synthase, which produces nitric oxide, a reactive free radical and a biologic mediator of several processes (Lauridsen, Olesen, 2017). Other molecules, such as leptin and neuropeptide Y (NPY) can also play a key role in the pathogenesis of obesity and depression { Yang, 2016 #6493, Lu and Ho, 2016, Lu et al., 2015). Neurons containing leptin receptors in the lateral hypothalamic area mediate leptin action on the mesolimbic system and contribute to the control of energy balance (Leinninger et al., 2011). Similarly, NPY acts on mesolimbic dopamine circuits to increase motivated behaviors toward food (West and Roseberry, 2017). In addition, the use of psychotropic medications, which have well-documented effects on the dopamine system, have also been reported to affect peripheral and central glucose/insulin metabolism, both directly through actions on glucose and insulin pathways and indirectly through behavioral/lifestyle effects (e.g. eating and activity patterns) (Contreras-Shannon et al., 2013, Heal et al., 2012, Khoza and Barner, 2011, Palmiere et al., 2017).

It has been suggested, based on the extant findings, that the subgroup of individuals with a mental disorder and obesity (and/or metabolic comorbidities), represent a, at least partially, distinct subtype (Mansur, Brietzke, 2015, Veltman, Lamers, 2017). This concept lacks sufficient empirical data for validation; similarly, the putative biological mechanisms underlying it are not yet well-established. Nonetheless, results described herein of vulnerable subgroups (i.e. obesity), integrated with biologically plausible substrates (i.e. insulin and dopamine signaling) should substantiate future research efforts.

We reported changes in gene expression and co-expression of multiple genes. However, we did not directly measure dopamine or insulin bioactivity, which is needed to interpret and contextualize these findings. Gene expression may be a proxy of, but it is not a direct measurement of protein levels and activity. Microarray analysis, as used herein, does not allow for the interrogation of alternative splicing products/different transcripts of the same gene. Different isoforms could have different effects; therefore the discussion of the biological meaning of these findings is somehow limited. Moreover, there was no validation with quantitative polymerase chain reaction (qPCR), nor can we infer the cell-type specificity of our findings (neurons, astrocytes, oligodendrocytes, or microglial cells). Even when assuming that our results are specific for neurons, there is no additional information from cellular location within neurons (i.e. terminals, dendrites and cell bodies; pre or post-synapsis), which are known to influence dopamine and insulin signaling (Purves-Tyson, Owens, 2017). There was no detailed information on clinical history; therefore we could not assess potential relevant confounding factors (e.g. sedentary lifestyle) and we cannot make inferences on behavioral or symptomatic manifestations. This is also a cross-sectional study, therefore causal, direct/indirect and/or compensatory processes cannot be conclusively established.

5. Conclusions

In conclusion, we documented, in individuals with SCZ, BD and MDD, changes in expression dopamine- and insulin-regulating genes in two key brain regions that are implicated in the pathophysiology of mood and psychotic disorders. Our results suggest that the differential expression of dopamine-related molecules in individuals with mental disorders is related to altered expression of insulin signaling genes; and that obesity has region-specific moderating effects on gene expression and co-expression, supporting the hypothesis that metabolic systems are critical mediators of dopaminergic function. The interface between dopamine and insulin metabolism represents a unique opportunity to take a transdisciplinary approach to refine pathogenic models of mental disorders, and possibly substantiate the development of disease modifying treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Expressions of dopamine and insulin genes in the dlPFC were moderated by BMI
- In the hippocampus, we observed lower expression of dopamine- and insulin-related genes
- Illnesses by BMI effects on dopamine genes in the dlPFC were mediated by INSR
- IRS1/2 fully mediated the effects of illnesses on the hippocampal dopamine genes

Figure 1–.

Gene Expression in the Dorsolateral Prefrontal Cortex. Mean standardized expression values of insulin and dopamine signaling genes in the dorsolateral prefrontal cortex, according to group (HC vs. MI) and BMI. For illustration purposes, BMI was dichotomized as obesity (BMI ≥ 30 kg/m²) and non-obesity (BMI < 30 kg/m²). HC: healthy controls; MI: mental illness.

Table 1–**Sample Characteristics**

	dlPFC sample			Hippocampus sample		
	Healthy Controls(n = 209)	Mental Illnesses (n = 321)	p-value	Healthy Controls (n = 180)	Mental Illnesses (n = 196)	p-value
Age at death (years), mean (SD)	42.61 (13.39)	43.85 (11.98)	0.449 ^a	41.51 (13.42)	44.12 (11.63)	0.080 ^a
Gender (Female), n (%)	58 (27.8)	129 (40.2)	0.003 ^b	52 (28.9)	72 (36.7)	0.106 ^b
Race (Caucasian), n (%)	95 (45.5)	234 (72.9)	< 0.001 ^b	71 (39.4)	123 (62.8)	< 0.001 ^b
BMI (kg/m ²), mean (SD)	29.95 (6.78)	27.95 (5.71)	0.004 ^a	29.86 (6.77)	28.03 (6.22)	0.017 ^a
Obesity (BMI ≥ 30 kg/m ²), n (%)	87 (46.0)	76 (33.9)	0.012 ^b	81 (46.0)	71 (37.6)	0.101 ^b
PMI (hours), mean (SD)	30.51 (13.97)	31.83 (14.34)	0.245 ^a	30.19 (13.88)	32.46 (14.50)	0.097 ^a
Laboratorial Tests						
Ethanol, n (%)	9 (4.3)	52 (16.2)	< 0.001 ^b	8 (4.4)	35 (17.9)	< 0.001 ^b
Nicotine/Cotinine, n (%)	53 (25.4)	130 (40.5)	< 0.001 ^b	48 (26.7)	112 (57.1)	< 0.001 ^b
Benzodiazepines, n (%)	1 (0.5)	48 (15.0)	< 0.001 ^b	1 (0.6)	30 (15.3)	< 0.001 ^b
THC, n (%)	1 (0.5)	10 (3.3)	0.030 ^b	1 (0.6)	8 (4.3)	0.020 ^b
Cocaine, n (%)	1 (0.5)	14 (4.4)	0.008 ^b	1 (0.6)	11 (5.7)	0.005 ^b
Opiates, n (%)	9 (4.3)	48 (15.0)	< 0.001 ^b	6 (3.3)	28 (14.3)	< 0.001 ^b
Antipsychotics, n (%)	0 (0)	127 (39.7)	< 0.001 ^b	0 (0)	91 (46.7)	< 0.001 ^b
Antidepressants, n (%)	1 (0.7)	114 (35.5)	< 0.001 ^b	0 (0)	83 (42.3)	< 0.001 ^b
Mood Stabilizers, n (%)	1 (0.7)	63 (19.6)	< 0.001 ^b	0 (0)	41 (20.9)	< 0.001 ^b

dlPFC: dorsolateral prefrontal cortex; SD: standard deviation; BMI: body mass index; PMI: postmortem interval; THC: tetrahydrocannabinol

^a Mann-Whitney U;

^b Chi-square test

Table 2–

Parameter estimates from multivariate analysis of covariance (MANCOVA) tests, using 1,000 simple bootstrap samples with bias corrected and accelerated (BCa) 95% confidence intervals (CIs), adjusted for age, gender, race, ethanol and nicotine/cotinine.

	Main effects						Group (HC and MI) by BMI interactions		
	dlPFC			Hippocampus			dlPFC		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
INSR	−0.025	−0.151; 0.108	0.749	−0.164	−0.359; 0.017	0.103	−0.027	−0.045; −0.007	0.009
IRS1	−0.099	−0.309; 0.108	0.354	−0.423	−0.630; −0.220	0.001	−0.039	−0.065; −0.012	0.015
IRS2	−0.110	−0.296; 0.082	0.296	−0.270	−0.475; −0.079	0.010	−0.034	−0.059; −0.008	0.014
DDC	−0.116	−0.318; 0.084	0.280	−0.118	−0.297; 0.072	0.192	−0.036	−0.062; −0.009	0.023
TH	−0.153	−0.343; 0.062	0.137	−0.240	−0.431; −0.039	0.020	−0.025	−0.051; 0.006	0.095
VMAT2	−0.064	−0.272; 0.139	0.572	−0.168	−0.408; 0.074	0.115	−0.034	−0.062; −0.005	0.028
DRD1	−0.252	−0.449; −0.042	0.019	−0.325	−0.535; −0.113	0.005	−0.024	−0.051; 0.004	0.104
DRD2	0.021	−0.187; 0.234	0.860	−0.130	−0.347; 0.093	0.223	−0.030	−0.059; −0.001	0.047
DRD3	0.047	−0.133; 0.230	0.649	0.016	−0.211; 0.248	0.106	−0.023	−0.052; 0.006	0.117
DRD4	0.203	0.024; 0.393	0.027	−0.019	−0.243; 0.214	0.880	−0.016	−0.041; 0.011	0.216
DRD5	−0.166	−0.355; 0.038	0.124	−0.101	−0.340; 0.134	0.382	−0.034	−0.062; −0.004	0.030
COMT	0.074	−0.123; 0.273	0.482	−0.179	−0.402; 0.043	0.144	−0.012	−0.037; 0.016	0.387
DAT	−0.082	−0.270; 0.119	0.412	−0.035	−0.267; 0.184	0.110	−0.032	−0.058; −0.003	0.024
MAOA	0.390	0.196; 0.586	0.001	0.031	−0.179; 0.269	0.787	−0.014	−0.042; 0.015	0.349
MAOB	0.204	0.032; 0.389	0.042	−0.267	−0.490; −0.056	0.013	−0.011	−0.040; 0.019	0.470

HC: healthy controls; MI: mental illness; dlPFC: dorsolateral prefrontal cortex; CI: confidence interval

Table 3–

Moderated mediation models in the dorsolateral prefrontal cortex (dlPFC), adjusted for age, gender, race, ethanol and nicotine/cotinine. For all outcomes, path a_1 (MI to INSR) = 0.886*, path a_2 (BMI to INSR) = 0.009, path a_3 (MI * BMI to INSR) = -0.032*. Low BMI = 22.50 kg/m²; Moderate BMI = 28.80 kg/m²; High BMI = 35.11 kg/m².

Unstandardized path coefficients					
	INSR to outcome (path b_1)	INSR * BMI to outcome (path b_2)	MI to outcome (path c_1)	BMI to outcome (path c_2)	BMI * MI to outcome (path c_3)
DDC	0.469 *	0.020 *	-0.100	0.010	0.001
VMAT2	-0.080	0.037 **	-0.100	0.013	0.002
DRD2	0.595 *	0.010	0.111	0.013	-0.002
DRD5	0.591	-0.001	0.219	0.004	-0.013
DAT	0.580 *	0.012	0.022	0.007	-0.003

Conditional indirect effects of MI on the outcomes at values of the moderator (BMI).				
	BMI	Effect	SE	95% CI
DDC	Low	0.159	0.101	-0.051; 0.349
	Moderate	0.026	0.078	-0.179; 0.129
	High	-0.259	0.110	-0.472; -0.045
VMAT2	Low	0.062	0.083	-0.042; 0.290
	Moderate	-0.025	0.076	-0.175; 0.122
	High	-0.276	0.121	-0.523; -0.049
DRD2	Low	0.145	0.096	-0.037; 0.345
	Moderate	-0.022	0.067	-0.155; 0.110
	High	-0.215	0.094	-0.412; -0.041
DRD5	Low	0.098	0.063	-0.030; 0.222
	Moderate	-0.014	0.043	-0.099; 0.067
	High	-0.122	0.058	-0.260; -0.028
DAT	Low	0.125	0.092	-0.057; 0.306
	Moderate	-0.031	0.071	-0.164; 0.114
	High	-0.214	0.097	-0.410; -0.024

* $p < 0.05$;

** $p < 0.001$. BMI: body mass index; MI: mental illness; SE: standard error; CI: confidence interval

Table 4–

Mediation models in the hippocampus, adjusted for age, gender, race, ethanol and nicotine/cotinine. For all outcomes, path a_1 (MI to IRS1) = -0.368^* and path a_2 (MI to IRS2) = -0.245^* .

Unstandardized path coefficients						
	IRS1 to outcome (path b ₁)		IRS2 to outcome (path b ₂)	Direct effect of MI (path c')		Total effect outcome on
TH	0.100		0.218 *	-0.148		-0.238 *
DRD1	0.210 *		0.342 **	-0.141		-0.305 *
MAOB	0.341 **		0.298 **	-0.040		-0.239 *
Indirect effects						
	IRS1			IRS2		
	Indirect effect	SE	95% CI	Indirect effect	SE	95% CI
TH	-0.037	0.029	-0.108; 0.008	-0.053	0.033	-0.145; -0.009
DRD1	-0.081	0.038	-0.176; -0.025	-0.083	0.040	-0.176; -0.018
MAOB	-0.125	0.044	-0.321; -0.071	-0.073	0.035	-0.154; -0.018

* $p < 0.05$;

** $p < 0.001$. MI: mental illness; SE: standard error; CI: confidence interval