Published in final edited form as:

Psychosom Med. 2012 June ; 74(5): 476–482. doi:10.1097/PSY.0b013e31824d0865.

A Neural Circuitry Linking Insulin Resistance to Depressed Mood

John P. Ryan, Ph.D.¹, Lei K. Sheu, Ph.D.¹, Hugo D. Critchley, D.Phil.^{2,3,4}, and Peter J. Gianaros, Ph.D.^{5,1}

¹Department of Psychiatry, University of Pittsburgh

²Brighton and Sussex Medical School, University of Sussex

³Sackler Centre for Consciousness Science, University of Sussex

⁴Sussex Partnership NHS Foundation Trust, UK

⁵Department of Psychology, University of Pittsburgh

Abstract

OBJECTIVE—Insulin resistance (IR) confers risk for Type 2 Diabetes and is associated with depressed mood. Neurons within ventral striatum (VS) are sensitive to insulin levels, and show altered function in the context of both IR and depression. Hence, VS may represent a critical component of a neural circuitry linking IR to depressed mood.

METHOD—Ninety adults (aged 30–50 years) free from major psychiatric illnesses and diabetes participated. Fasting blood was sampled, and participants completed a set of questionnaires (including the Beck Depression Inventory-II). Participants also underwent resting-state functional magnetic resonance imaging of the brain. Seed-based connectivity analyses, centered on VS, were conducted to examine how resting inter-regional connectivity patterns covaried with IR and depressed mood.

RESULTS—Higher levels of IR covaried with increased connective strength between the left VS and two regions: Insula and anterior mid-cingulate cortex (aMCC). Moreover, aMCC-VS connectivity predicted depressed mood, $b = 0.93$, $se = .36$, $F_{change}(1, 81) = 6.54$, $p = 0.01$. Finally, aMCC-VS connectivity was shown by Monte Carlo analysis to mediate the relationship between IR and depressed mood, $a*b$ indirect effect = 0.16, $CI = 0.005 - 0.39$, $p = 0.03$.

CONCLUSIONS—IR relates to changes in the functional connectivity between VS and aMCC. These changes in inter-regional communication partly account for the coupling of IR to depressed mood in otherwise healthy adults. These findings are relevant for understanding bidirectional associations between diabetes risk and depressed mood.

Keywords

anterior cingulate cortex; depressed mood; insulin resistance; ventral striatum; insula; fMRI

Address correspondence to: John P. Ryan, Ph.D., 3811 O'Hara St., Pittsburgh, PA 15213, ryanjp@upmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Financial Disclosures: None

Drs. Gianaros, Ryan, Sheu and Critchley have no competing interests.

Introduction

More than 20 million people in the United States have Type 2 Diabetes (T2D), a disease of glucose dysregulation that increases risk for heart disease, stroke, blindness, and autonomic neuropathy (1). A precursor to T2D is insulin resistance (IR)—a condition in which cells in the liver, muscle, fat and brain lose sensitivity to insulin. As sensitivity to insulin decreases, the pancreas secretes more insulin to compensate for this reduced sensitivity, resulting in elevated fasting insulin levels. Of clinical importance, IR doubles T2D risk (2) and is comorbid with depressive symptoms (3). A recent meta-analysis found that ~1 in 5 people with T2D meet diagnostic criteria for depression (4).

Central nervous system IR associates with central fat deposition (or truncal adiposity), and is linked to premature cognitive impairments (5,6). It is noteworthy that several brain regions are not only sensitive to insulin, but are also implicated in the pathophysiology of depression. One such region is the ventral striatum (VS). The VS encompasses the nucleus accumbens and globus pallidus, as well as adjacent portions of the putamen and ventral caudate nucleus (7–10). In a study examining glucose metabolism using positron emission tomography, insulin administration increased glucose metabolism in the VS and insula (11). However, increased glucose metabolism within the VS was attenuated in individuals with IR (8). This finding suggests that insulin sensitivity is regionally specific within the brain, and that the effects of insulin (and IR) may be pronounced in regions implicated in eating and other consummatory behaviors (insula) and appetitive motivation (VS).

The VS has specific anatomical connectivity with orbitofrontal and anterior cingulate cortices (ACC) (12), and insula (13). Moreover, dysfunction of the basal ganglia – cingulate circuitry is increasingly recognized to be a common feature of mood disorders (14). Hence, the VS of the basal ganglia is hypoactive in individuals with depression (15,16), and stimulation of the VS can alleviate depressive symptoms (17,18). Further, a region within ACC has been identified as a “hub” for alterations in the task-independent functionality of so-called ‘resting state’ brain networks, particularly in depression. Hence, depressed individuals exhibit characteristic changes in resting state connectivity patterns (described below), including stronger connectivity between the dorsal ACC and anatomically linked regions that are implicated in cognitive control, affective processing, and self-representation (16). Moreover, enhanced ACC connectivity has been related to depressive symptom severity (19). These findings highlight the importance of functional relationships *between* brain areas during resting states to understanding the expression of, and risk for, psychopathologies of mood.

To elaborate, whereas many fMRI studies utilize paradigms in which brain activity is examined across different experimental conditions of a given task, growing research in the past decade has recognized the utility of examining patterns of network activity in the brain while individuals are instructed to remain awake, but rest quietly. Methodological advances for measuring functional networks at rest within the brain, including quantitative study of functional connectivity from low frequency fluctuations in the blood-oxygen level-dependent (BOLD) signal, can thus be applied to quantify integrated relationships across neural regions (20). Several methods are available to examine such resting state brain network activity (21), including seed-based connectivity analysis. The advantage of seed-based connectivity analysis is its ability to quantify time-varying correlations between an *a priori* region and all other brain regions simultaneously. By this approach, a “seed region” is selected, and the MRI time-series signal is extracted from that region. The signal is then correlated with all voxels in the brain to identify how activity in particular regions correlates with seed region activity. These correlations can then be examined in relation to a third variable to test whether the third variable associates with the degree of connectivity between

the seed region and other regions. Finally, resting state activity recorded over several minutes affords an opportunity to quantify how ‘intrinsic’ functional connectivity within the brain covaries with a physiological risk factor, such as IR.

Given the centrality of the VS in both depression and sensitivity to insulin, the VS was selected as the seed region for the present study. Using resting-state fMRI data, functional connectivity of the VS seed was calculated from time-dependent correlations in signal amplitude across brain regions. Next examined was how functional connectivity with the VS associated with individual differences in IR and self-reported depressed mood. We selectively examined VS connectivity with regions involved in reward processing and with known striatal anatomical connectivity, including the ACC, insula, gyrus rectus, and middle and inferior frontal gyri. The overall question addressed was whether VS connectivity covaries with IR, and whether such covariation might partly explain individual differences in depressed mood. We hypothesized that IR would associate with alterations in VS connectivity, particularly with regions involved in depressed mood, such as the ACC, and that these alterations would explain inter-individual variation in depressed mood.

Methods and Materials

Participants

Participants were recruited by mass mailings to residents of Allegheny County, Pennsylvania, USA. Respondents were screened to exclude those with (i) a self-reported history of cardiovascular disease (including treatment for or diagnoses of hypertension, stroke, myocardial infarction, congestive heart failure, and atrial or ventricular arrhythmias); (ii) prior cardiovascular surgery (including coronary bypass, carotid artery, or peripheral vascular surgery); (iii) chronic kidney or liver conditions, diagnosed Type I/II diabetes, or any pulmonary or respiratory disease; (iv) self-reported current or past diagnoses of a substance abuse or mood disorder (including alcohol dependence, a somatization disorder, major depression, and panic or other anxiety disorders), as confirmed on interview using the Patient Health Questionnaire (22), an inventory validated in outpatient (23–25) and community samples (26) for sensitivity and specificity against the Diagnostic and Statistical Manual of Mental Disorders IV; (v) prior cerebrovascular trauma involving loss of consciousness; (vi) prior neurosurgery or any neurological condition; (vii) being pregnant (verified by urine test in females); (viii) having claustrophobia or metallic implants; or (ix) taking psychotropic, lipid lowering, or cardiovascular medications. After providing a description of the study, written informed consent was obtained from all participants. The University of Pittsburgh Institutional Review Board approved all study procedures.

One hundred and fifty-five participants completed the study between 2008 and 2011. Nineteen participants had poor quality resting state MRI data (e.g., signal loss, excessive movement artifacts, etc.), and were excluded from further analysis. Of the remaining 136 participants, three had incomplete blood data, and 43 had fasting insulin levels below detectable levels. These participants were not included in the primary analyses examining the relationship of functional connectivity to IR. However, ancillary analyses examining differences between those with and without detectable insulin levels were performed (see Results). Participants with undetectable fasting insulin levels had a smaller waist circumference ($t [134] = 4.15, p < 0.001$), lower triglyceride levels ($t [132] = 3.44, p < 0.001$), and higher HDL ($t [132] = 2.74, p = 0.006$) relative to those with detectable insulin levels. There were no significant differences between these groups in sex, age, LDL, depression or anxiety scores, and the fasting glucose levels trended toward being lower in participants with undetectable insulin levels, $t (132) = 1.62, p = 0.10$. The final sample size for primary analyses thus included 90 participants (46 men; 30–50 years old; participant characteristics are in Table 1).

Blood Samples and Psychometric Questionnaires

Participants completed a fasting blood draw prior to MRI. Serum was analyzed using a Synchron CX chemistry analyzer (Beckman-Coulter, Brea, CA) and associated reagents (GLU, TG, HDLD, CHOL) for glucose, triglyceride, HDL and total cholesterol. LDL cholesterol values were estimated by subtracting the HDL cholesterol level from total cholesterol. Insulin was quantified using the Immulite Immunoassay System (Siemens). Insulin resistance (IR) was computed using Homeostatic Model Assessment (HOMA) values – which approximate IR based on fasting glucose and insulin levels $((\text{glucose} \times \text{insulin}) / 405)$ (27). In a session prior to MRI, participants completed a packet of questionnaires, including the Beck Depression Inventory (BDI) II (28). The BDI-II is a 21-item questionnaire, with higher scores indicating a more depressed mood. The BDI-II for our sample had acceptable internal consistency, $\alpha=0.73$. Insulin, HOMA-IR and BDI-II scores were logtransformed to achieve normal distributions before analysis.

Image Acquisition

Approximately 4 weeks separated the initial visit involving questionnaire completion and the MRI visit. Resting state fMRI data were acquired on a 3 Tesla Trio TIM whole-body scanner (Siemens, Erlangen, Germany), equipped with a 12-channel, phased-array head coil. Resting state images were acquired with a gradient-echo EPI sequence (FOV=205×205 mm; 64×64 matrix; TR=2 sec; TE=28 ms; flip angle=90°). During scanning, participants' ears were shielded from noise, but they were allowed to keep their eyes open while resting quietly. Thirty-nine sections (3mm thick, no gap) were obtained sequentially, yielding 150 BOLD images (the first 3 images were discarded, allowing for magnetic equilibration). Anatomical images used for functional image co-registration and normalization were collected using a T₁-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence (FOV=256×208 mm; 256×208 mm matrix; TR=2100 ms; inversion time=1100 ms; TE=3.29 ms; flip angle=8°; 192 sections; 1mm thick, no gap).

Image Processing

Resting BOLD images were preprocessed using SPM8 (Wellcome Trust Centre for Neuroimaging; <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>). BOLD images were realigned to the first series image, co-registered to the MPRAGE, and normalized to the ICBM/MNI space using the SPM template. Normalized images were smoothed with a 6-mm FWHM isotropic Gaussian kernel and re-sliced to 2×2×2 mm voxels. Connectivity images were constructed using a seed approach (29–31). Before analysis, the whole-brain resting BOLD signals were filtered with the SPM high-pass filter of 100 sec to remove signals with frequencies <0.01HZ, while high-frequency signals (>0.1HZ) were filtered by an autoregressive model. As such, only conventional resting state signals (0.01–0.1 Hz) were included. The mean BOLD signal time series was extracted from a 4-mm sphere in bilateral VS (x,y,z = ±9, 8, 9), based on coordinates from previous research on striatum functional connectivity (32). The VS seed was the only seed analyzed because of our hypotheses of this region showing functional overlap in the context of *both* insulin sensitivity *and* depressed mood. Each seed time-series was meancentered, adjusted for outliers, and entered as a regressor in individual general linear model (GLM) design matrices. Nuisance regressors, including a time series from the 4th ventricle (5mm sphere, x,y,z = 0, -43, -26) and an averaged time series extracted from a bilateral white matter seed (2mm sphere, x,y,z = ±26, 8, 36), were also included to control for confounding noise. Movement parameters obtained from realignment were also entered to the GLM to account for head motion. Second-level, multiple regressions were conducted using log-adjusted HOMA-IR values as the predictor of interest, with sex and age as covariates.

A region-of-interest (ROI) mask was created with MarsBaR and used for planned analyses. Anatomical boundaries were defined using Automated Anatomical Labeling masks for the MNI brain (33), and Brodmann areas were selected from the Talairach Daemon database within WFU_PickAtlas (34,35). This ROI included BA10, 11, 12, 13, 25, 32, bilateral insula, ACC, gyrus rectus, and middle and inferior frontal gyri. These regions were selected for mask inclusion because of their relevance to appetitive, consummatory, and motivational processing (12,36), and because of our a priori interest on areas networked with VS. AlphaSim software (37) was used to determine the appropriate correction threshold for the ROI mask ($p = .005$, voxel size = 2mm, 5000 iterations) using the appropriate FWHM values for each analysis.

Data Extraction and Mediation

Connectivity values were computed by the eigenvariate extraction option for each cluster of VS connectivity. This method extracts the first eigenvariate of the cluster for each participant, and these values were imported into SPSS (v.20, IBM Inc., Armonk, NY). Hierarchical regression models were then run to determine regression coefficients after controlling for covariates. In the first step, covariates were entered, followed by the predictor of interest in the second step. Testing of the indirect effect ($a*b$) was conducted with an online Monte Carlo calculator (<http://www.people.ku.edu/~preacher/medmc/medmc.htm>). Eigenvariate (connectivity) values were regressed onto covariates and the predictor of interest in SPSS, yielding path coefficients (“a” and “b”) representing unstandardized regression coefficients. Path coefficients and standard errors were tested with 100,000 repetitions and 95% confidence intervals to test indirect ($a*b$) effects.

Results

Connectivity and IR

Higher HOMA-IR values covaried with a stronger connectivity between the left VS and two regions: the left anterior insula (BA13 $x,y,z = -38, -6, 4$; $k = 461$; peak $T = 4.36$), and the dorsal portion of the left anterior mid-cingulate cortex (aMCC), following the nomenclature of Vogt (38) (BA 32; $x,y,z = -14, 42, 18$; $k = 281$; peak $T = 4.37$) (see Figure 1). At the same corrected statistical threshold, no brain regions showed altered connectivity with the right VS seed, even at a more lenient statistical threshold ($k = 104$, $p = .005$ corresponding to a threshold of $p = 0.20$), and no regions showed negative correlations with HOMA-IR values.

Connectivity and Depressed Mood

To test if left VS connectivity related to depressed mood, as well as to account for covariates, connectivity values were extracted and analyzed in hierarchical regression models (high- and low-density lipoproteins, triglycerides, waist circumference, age and sex entered in the first step; predictor of interest in the second step). Connectivity values were extracted using the eigenvariate option in SPM8. The relationship between HOMA-IR values and aMCC-VS connectivity persisted after accounting for high- and low-density lipoproteins, triglycerides, waist circumference, age and sex, $b = 0.17$, $se = 0.07$, $F_{change}(1, 82) = 5.05$, $p = 0.03$, $R^2_{change} = 0.05$. Also, after accounting for HOMA-IR values, in addition to the above covariates, aMCC-VS connectivity predicted depressed mood, $b = 0.93$, $se = 0.36$, $F_{change}(1, 81) = 6.54$, $p = 0.01$, $R^2_{change} = 0.07$.

Again, the relationship between HOMA-IR and insula-VS connectivity was significant after accounting for all covariates ($b = 0.26$, $se = 0.09$, $F_{change}[1, 82] = 8.30$, $p = 0.005$, $R^2_{change} = 0.08$), but insula-VS connectivity did not predict BDI-II scores, $b = 0.34$, $se = 0.31$;

$F_{change}(1, 81) = 1.18, p = 0.28, R^2_{change} = 0.01$. Accordingly, mediation tests were not performed for insula-VS connectivity.

After controlling for sex, age, LDL, HDL, triglycerides and waist circumference, HOMA-IR values covaried positively with depressed mood, but this association did not reach statistical significance, $b = 0.35, se = 0.26, p = 0.17$.

Mediation

To test for indirect mediation by aMCC-VS connectivity, a Monte Carlo analysis was performed on the indirect effect (39) of HOMA-IR $\rightarrow$ aMCC-VS connectivity $\rightarrow$ BDI-II, which revealed shared variance between HOMA-IR and depressed mood, $a*b = 0.16, CI = 0.005 - 0.39, p = 0.03$ (Figure 2).

Alternate Analyses

Research (40) has demonstrated the existence of BDI-II sub-factors, namely, cognitive/affective and somatic sub-factors. Hence, we explored whether the aMCC-VS connectivity related to these BDI-II sub-factors. Interestingly, after controlling for HOMA-IR values, as well as the above covariates, aMCC-VS connectivity predicted both log-transformed somatic scores ($b = 0.55, se = 0.27, p = 0.04$), and log-transformed cognitive/affective scores ($b = 0.81, se = 0.35, p = 0.02$). BDI-II items comprising the cognitive/affective sub-factor include anhedonic symptoms, including loss of pleasure, sadness, and feelings of worthlessness. The somatic items, however, associate primarily with vegetative functions (appetite, sleep, and fatigue) that would appear to relate to motivational drive. In contrast to connectivity between aMCC and VS, insula-VS connectivity did not relate to either sub-factor ($bs < 0.33, ps > 0.28$).

To test the independence of aMCC-VS connectivity mediating HOMA-IR and depressed mood from anxiety, trait anxiety scores from the Spielberger State-Trait Anxiety Inventory (STAI) (41) were included as a covariate in ancillary analyses. Although *state* anxiety levels were not assessed, *Trait* anxiety scores may be better suited to this analysis due to the delay in time between completion of the questionnaires and MRI, as well as the joint administration of the BDI-II and STAI. HOMA-IR continued to predict aMCC-VS connectivity ($b = 0.16, se = 0.08, p = 0.04$), and aMCC-VS connectivity predicted BDI-II scores after covarying HOMA-IR, $b = 0.64, se = 0.30, p = 0.03$. The indirect effect was reduced, and trended toward significance, $a*b = 0.10, CI = -0.001 - 0.27, p = 0.08$.

Given the above, we explored the relationship between trait anxiety, HOMA-IR, and resting state connectivity. Regressing trait anxiety on HOMA-IR values after controlling for covariates (LDL, HDL, triglycerides, sex, age and waist circumference) was not significant, $F_{change}(1,82) = 1.53, p = 0.21$. If depressed mood scores were entered as an additional covariate, significance was reduced further, $F_{change}(1,81) = 0.24, p = 0.62$. Neither of the clusters of connectivity that associated with HOMA-IR were themselves associated with trait anxiety: the association between trait anxiety and VS-insula connectivity was not significant, $F_{change}(1,82) = 0.12, p = 0.73$, nor was the association between trait anxiety and VS-aMCC connectivity, $F_{change}(1,82) = 2.49, p = 0.12$. Finally, in comparability to the HOMA-IR findings, the magnitude of these associations were reduced further if BDI-II values were included as a covariate (e.g. VS-aMCC $F_{change}[1,81] = 0.03, p = 0.86$).

An alternative possibility, is that rather than HOMA-IR accounting for strengthened aMCC-VS connectivity, *fasting glucose* levels could account for individual differences in connectivity. Yet, additional analyses did not favor this possibility. Hence, fasting glucose did not predict aMCC-VS connectivity, after controlling for covariates, $b = 0.00, se = 0.00, p = 0.94$. Consistent with the interpretation of the effects being related to HOMA-IR, fasting

insulin (log transformed) predicted aMCC-VS connectivity ($b = 0.10$, $se = 0.05$, $p = 0.04$), and connectivity continued to predict BDI-II scores after covarying for fasting insulin, $b = 0.92$, $se = 0.36$, $p = 0.01$. Finally, the indirect effect persisted: $a*b = 0.09$, $CI = 0.001 - 0.23$, $p = 0.06$.

We tested for differences in brain connectivity between those with detectable fasting insulin levels and those without to assess for sampling bias. In a whole-brain voxelwise analysis ($p < 0.05$ FWE) no clusters were significantly different between groups. Utilizing the ROI mask from above with the appropriate threshold ($p = .005$, $k = 132$) also yielded no regions of VS connectivity that differed between groups.

Discussion

Alterations in the functional connectivity between the left aMCC and left VS mediated the relationship between HOMA-IR and symptoms of depressed mood, suggesting that the functional interplay between the cingulate cortex and basal ganglia may be involved in mood alterations often observed in those with or at risk for T2D. Noteworthy is that participants studied were not severely depressed (BDI-II scores ranged from 0 to 14) and had no reported history of a diabetic diagnosis. Thus, the present preclinical observations would appear to be un-confounded by current treatment regimens, as well as by awareness of disease diagnoses.

The present observations add to a growing literature documenting alterations in striatal regions of basal ganglia circuits that associate with disruptions in glucose regulation. One of the first demonstrations of the relevance of the basal ganglia for IR showed that peripheral infusions of insulin, during suppression of endogenous insulin via somatostatin, increased the metabolic rate in the VS, but this effect was blunted in IR individuals (11). Although our study did not examine metabolic rate, it extends this finding to show how the connectivity of the VS within the basal ganglia is altered in conjunction with IR. Future studies should attempt to disentangle the causal relationship between alterations in insulin sensitivity and changes in basal ganglia functionality that could result from or precede these changes.

A separate literature has started to reveal alterations in brain structure that occur in the context of T2D. In a study utilizing magnetization transfer ratios, Kumar and colleagues found alterations in the structure of the caudate nucleus in patients with comorbid depression and T2D (42). In a whole-brain examination of grey matter, individuals with T2D had smaller grey matter volumes in ACC and orbitofrontal regions (43). Although these studies did not find structural alterations in VS regions, they highlight the centrality of nodes within the so-called 'reward' network, which encompasses orbitofrontal cortex and caudate, in brain alterations that accompany T2D.

Previous work has demonstrated anatomical and functional connectivity between the VS and ACC, first in trans-neuronal tracing studies done in animal models (12) and also in human imaging studies (32). By using a seed region in the VS, we demonstrate that the increased connectivity with the aMCC was associated with increased depressed mood. In this regard, it is noteworthy that an earlier study in a smaller sample of individuals failed to identify differences in the connectivity between the VS and ACC between individuals with clinical depression and controls, a null finding that seems divergent from our observations (19). We note, however, that we studied a non-clinical sample and did not employ a case-control design. Further, the earlier study employed a whole-brain rather than seed-based methodology, and we examined variation in a *continuous* measure of depressive symptomatology in a larger nonclinical sample. Such methodological differences could in part account for apparent differences in study findings.

The laterality of the effects, namely that only left VS connectivity covaried with HOMA-IR, was unexpected. Accordingly, explanations for the lack of findings with the right VS seed are speculative. Moreover, in a cross-sectional design, it is unfeasible to examine temporally ordered changes in brain connectivity as insulin sensitivity decreases. Future longitudinal studies could perhaps determine such changes that differentiate the response of the left and right striatum as IR progresses. Further, it should be emphasized again that the current sample met neither criteria for T2D nor depression, and the pattern of results differ (or be more pronounced) in a sample with IR that has progressed closer to the clinical horizons of these syndromes.

More broadly, our data may be relevant to understanding interactions between dopamine dysfunction, psychological wellbeing, and the over-expression of metabolic syndrome and diabetes in patients with psychosis and/or those treated with dopamine receptor antagonists. Based on known relationships between reward processing, dopamine, and food intake (44), the present study supports a model in which functional alterations in reward circuits vary directly with IR levels across individuals. Due to the cross-sectional nature of the present study, however, we can only speculate on the longer-term temporal dynamics of the relationship between IR and reward circuit alterations. Yet, because of the population tested here, we believe our observations highlight the potential of neuroimaging to identify neural correlates of *risk* that may occur early in the natural history of T2D. Future studies will need to integrate prospective measurements of disease progression to better understand the temporal relationships of brain network alterations to peripheral metabolic changes.

In addition to the enhanced coupling between aMCC-VS, we also observed stronger functional connectivity between left VS and insula in those with higher HOMA-IR levels. These findings are clinically relevant when considered in the context of insula function and reward-related addictive behaviors. Hence, focal insula lesions eliminate psychological cravings of reinforcing stimuli through disruptions of visceral reward-dependent motivational signaling (a factor relevant to associations between IR, food intake, and appetitive behaviors)(45). Although future study is warranted to replicate and extend these findings, they agree with obesity research suggesting that eating to excess may share neural circuitries or pathways of drug addiction. Indeed, youth at risk for obesity exhibit enhanced reactivity within reward circuits to tasting palatable food (46). In parallel, Stice and colleagues have proposed a model in which hyper-functioning of the gustatory cortex (located within the insula and adjacent operculum) and enhanced experiences of pleasurable reward from eating may lead to down-regulation of dopamine receptors in striatal pathways (47). Finally, recent work has demonstrated alterations in resting state networks in conjunction with IR, particularly in networks that include regions involved in eating behaviors and reward processing (48). The present study extends this work to show that these alterations may aid in understanding the neurobiology of mood alterations that commonly co-occur with physical diseases, such as T2D.

The present findings also extend a growing literature on the relationship between IR, T2D and depressive disorders. Although some studies report no relationship between IR and major depression in non-diabetic young adults (49), others demonstrate cross-sectional associations (50). In our sample, the bivariate relationship between HOMA-IR and BDI-II scores was not significant after controlling for various metabolic covariates ($p = 0.17$), although the indirect effect of HOMA-IR $\rightarrow$ aMCC-VS connectivity $\rightarrow$ BDI-II scores was significant. Given the subclinical status of these participants, both on HOMA measures of IR and BDI-II measures of depressed mood, it is perhaps not surprising that the relationship was not significant. However, it has been argued recently that the significance of direct paths (e.g., from IR to depressed mood) is not a formal requirement for mediation (51,52). Viewed in this context, the present results indicate that even in the absence of the direct

effect, the connectivity of the aMCC to the VS accounts for a significant portion in shared variance between IR and depressed mood, and they highlight the importance of examining neurobiological circuitries and pathways for understanding the comorbidity of these disorders.

Although the methodology utilized in this study does not permit directional inferences regarding causation (i.e., effective connectivity) between functional changes across brain regions, primate anatomical studies suggest that the VS receives dense input from ACC, and this input is largely unidirectional (with feedback occurring via indirect thalamic pathways) (12). Future studies utilizing effective connectivity methods may be able to parse the nature of the relationship between aMCC and VS that associates with depressive symptoms, as well as the functional importance of intermediary (e.g., thalamic) relay nodes. Furthermore, this study is cross-sectional. Thus, causal inferences are not supported regarding the relationships between IR, brain connectivity, and depressed mood. As noted, however, IR and depression associations at the epidemiological level are bidirectional, conferring risk for each other through a presumptive shared pathway. The connectivity between the ACC and VS may represent one such pathway. Finally, a disadvantage of seed-based connectivity is that connectivity is only determined for the seed region. It is possible that additional brain regions display altered connectivity – just not with the VS – in association with IR, and these may contribute to depressed mood. Future studies utilizing whole-brain connectivity methods, such as independent components analysis, would be able to address such issues (53).

There are several other limitations to the present study. First, a formal structured clinical interview was not administered. Potential current or past psychiatric diagnoses were inferred only from self-report instruments. It is possible that past events of mental illness were not diagnosed or detected, and therefore not self-reported. Individuals with a prior history of depressive episodes exhibit altered functional connectivity after remission (54), and it is possible that our sample may have included such individuals. Therefore, the associations reported here could be different in samples screened with structured clinical interviews, in clinical samples, and in those with established T2D.

Next, a portion of the original sample had undetectable insulin levels, and were thus excluded from analysis. These individuals had smaller waist circumferences, lower triglyceride levels, and higher HDL levels relative to those included in the present analyses. The participants who had detectable insulin levels had an average BMI of 28, which is overweight. Waist circumference was included as a covariate to attempt to examine IR independent of variance shared with adiposity; however, we acknowledge that our sample may not be representative of those who are either obese or of normal weight. However, group comparisons between those with and without detectable fasting insulin showed no differences in connectivity between the VS and other brain regions. Recent work (46) has begun to investigate the associations between body mass index and resting state functional connectivity, but longitudinal studies will be necessary to investigate if these networks change as individuals move from overweight to obese.

Finally, the present study examined spontaneous brain activity while participants rested passively, and it also relied on a fasting blood sample to quantify IR. There are numerous future directions that would enable a more comprehensive understanding of the functional alterations that occur in the brain in conjunction with IR. For example, future studies could include tasks that more selectively probe emotional processing or regulatory alterations that underlie depressive mood, as well as manipulate physiological parameters such as prandial state to examine how metabolic challenges affect brain function related to mood processes. It would also appear important to evaluate the length of exposure to IR to determine possible

chronicity effects. Additionally, the present study examined only the relationships between functional brain activity, as measured by the BOLD signal, and IR. Our observations, therefore, do not address the possible *structural* neural correlates of IR. Accordingly, future studies are needed to clarify whether volumetric changes in regional brain tissue or morphological changes in white matter tracts comprising distributed striatal circuitries relate to IR and depressed mood.

In summary, altered left aMCC-VS circuit function linked IR and depressed mood, leading to the predictions that such an alteration could (i) result from long-term insulin pathophysiology, or (ii) predispose individuals to reward-related behaviors promoting T2D risk accompanied by depressive mood. IR also associated with enhanced connectivity between insula and left VS in the present study, suggesting possible similarities with findings bearing on the neurobiology of addiction (55). These findings highlight the role of neurobiological circuitries for understanding the interplay between IR and mood, but further study is needed to clarify the causal relationships between these factors in the context of comorbid medical and psychological syndromes.

Acknowledgments

This work was supported by National Institutes of Health grants R01-HL089850, T32-HL00756, and F32-DK091962. This work was presented as a poster at the 2011 Annual Meeting of the American Psychosomatic Society.

List of Abbreviations

ACC	anterior cingulate cortex
aMCC	anterior mid-cingulate cortex
BDI-II	Beck Depression Inventory 2
HOMA-IR	Homeostatic Model Assessment
IR	Insulin Resistance
STAI	State-Trait Anxiety Inventory
T2D	Type 2 Diabetes
VS	Ventral Striatum

References

1. National Institute of Diabetes and Digestive and Kidney Diseases. [cited 2010 May 22] National Diabetes Statistics, 2007 fact sheet [Internet]. Available from: <http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm>
2. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB, Wilson PWF. Impact of Insulin Resistance on Risk of Type 2 Diabetes and Cardiovascular Disease in People With Metabolic Syndrome. *Diabetes Care*. 2007; 30:1219–1225. [PubMed: 17259468]
3. Adriaanse MC, Dekker JM, Nijpels G, Heine RJ, Snoek FJ, Pouwer F. Associations between depressive symptoms and insulin resistance: The Hoorn Study. *Diabetologia*. 2006; 49:2874–2877. [PubMed: 17066302]
4. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The Prevalence of Comorbid Depression in Adults With Diabetes. *Diabetes Care*. 2001; 24:1069–1078. [PubMed: 11375373]
5. Hallschmid M, Schultes B. Central nervous insulin resistance: a promising target in the treatment of metabolic and cognitive disorders? *Diabetologia*. 2009; 52:2264–2269. [PubMed: 19705099]

6. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology*. 1998; 50:164–168. [PubMed: 9443474]
7. Haber SN. The primate basal ganglia: parallel and integrative networks. *J Chem Neuroanat*. 2003; 26:317–330. [PubMed: 14729134]
8. Haber SN, Kim K-S, Mailly P, Calzavara R. Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentive-based learning. *J Neurosci*. 2006; 26:8368–8376. [PubMed: 16899732]
9. Delgado MR. Reward-related responses in the human striatum. *Ann N Y Acad Sci*. 2007; 1104:70–88. [PubMed: 17344522]
10. Voorn P, Vanderschuren LJMJ, Groenewegen HJ, Robbins TW, Pennartz CMA. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci*. 2004; 27:468–474. [PubMed: 15271494]
11. Anthony K, Reed LJ, Dunn JT, Bingham E, Hopkins D, Marsden PK, Amiel SA. Attenuation of Insulin-Evoked Responses in Brain Networks Controlling Appetite and Reward in Insulin Resistance. *Diabetes*. 2006; 55:2986–2992. [PubMed: 17065334]
12. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010; 35:4–26. [PubMed: 19812543]
13. Chikama M, McFarland NR, Amaral DG, Haber SN. Insular Cortical Projections to Functional Regions of the Striatum Correlate with Cortical Cytoarchitectonic Organization in the Primate. *J Neurosci*. 1997; 17:9686–9705. [PubMed: 9391023]
14. Middleton, FA. Cingulate Neurobiology and Disease. New York: Oxford; 2009. The contribution of anterior cingulate-basal ganglia circuitry to complex behavior and psychiatric disorders; p. 619–632.
15. Krishnan V, Nestler EJ. Linking molecules to mood: new insight into the biology of depression. *Am J Psychiatry*. 2010; 167:1305–1320. [PubMed: 20843874]
16. Savitz J, Drevets WC. Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neurosci Biobehav Rev*. 2009; 33:699–771. [PubMed: 19428491]
17. Bewernick BH, Hurlmann R, Matusch A, Kayser S, Grubert C, Hadrysiewicz B, Axmacher N, Lemke M, Cooper-Mahkorn D, Cohen MX, Brockmann H, Lenartz D, Sturm V, Schlaepfer TE. Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression. *Biol Psychiatry*. 2010; 67:110–116. [PubMed: 19914605]
18. Malone DA Jr, Dougherty DD, Rezai AR, Carpenter LL, Friehs GM, Eskandar EN, Rauch SL, Rasmussen SA, Machado AG, Kubu CS, Tyrka AR, Price LH, Stypulkowski PH, Giftakis JE, Rise MT, Malloy PF, Salloway SP, Greenberg BD. Deep brain stimulation of the ventral capsule/ventral striatum for treatment-resistant depression. *Biol Psychiatry*. 2009; 65:267–275. [PubMed: 18842257]
19. Sheline YI, Price JL, Yan Z, Mintun MA. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *Proc Natl Acad Sci USA*. 2010; 107:11020–11025. [PubMed: 20534464]
20. Greicius M, Krasnow B, Reiss A, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci USA*. 2003; 100:253–258. [PubMed: 12506194]
21. Margulies DS, Böttger J, Long X, Lv Y, Kelly C, Schäfer A, Goldhahn D, Abbushi A, Milham MP, Lohmann G, Villringer A. Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity. *MAGMA*. 2010; 23:289–307. [PubMed: 20972883]
22. Löwe B, Gräfe K, Zipfel S, Witte S, Loecherer B, Herzog W. Diagnosing ICD-10 depressive episodes: superior criterion validity of the Patient Health Questionnaire. *Psychother Psychosom*. 2004; 73:386–390. [PubMed: 15479995]
23. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *JAMA*. 1999; 282:1737–1744. [PubMed: 10568646]
24. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001; 16:606–613. [PubMed: 11556941]

25. Löwe B, Kroenke K, Herzog W, Gräfe K. Measuring depression outcome with a brief self-report instrument: sensitivity to change of the Patient Health Questionnaire (PHQ-9). *J Affect Disord.* 2004; 81:61–66. [PubMed: 15183601]
26. Martin A, Rief W, Klaiberg A, Braehler E. Validity of the Brief Patient Health Questionnaire Mood Scale (PHQ-9) in the general population. *Gen Hosp Psychiatry.* 2006; 28:71–77. [PubMed: 16377369]
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28:412–419. [PubMed: 3899825]
28. Beck, A.; Steer, RA.; Brown, GK. Manual for Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation; 1996.
29. Greicius M, Srivastava G, Reiss A, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci USA.* 2004; 101:4637–4642. [PubMed: 15070770]
30. Fox MD, Corbetta M, Snyder AZ, Vincent JL, Raichle ME. Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proc Natl Acad Sci USA.* 2006; 103:10046–10051. [PubMed: 16788060]
31. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci.* 2007; 8:700–711. [PubMed: 17704812]
32. Di Martino A, Scheres A, Margulies DS, Kelly AMC, Uddin LQ, Shehzad Z, et al. Functional Connectivity of Human Striatum: A Resting State fMRI Study. *Cerebral Cortex.* 2008; 18:2735–2747. [PubMed: 18400794]
33. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* 2002; 15:273–289. [PubMed: 11771995]
34. Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, Kochunov PV, Nickerson D, Mikiten SA, Fox PT. Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp.* 2000; 10:120–131. [PubMed: 10912591]
35. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage.* 2003; 19:1233–1239. [PubMed: 12880848]
36. Rolls ET. Functions of the orbitofrontal and pregenual cingulate cortex in taste, olfaction, appetite and emotion. *Acta Physiol Hung.* 2008; 95:131–164. [PubMed: 18642756]
37. Ward BD. 2000 Available from: <http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf>.
38. Vogt, BA. Cingulate Neurobiology and Disease. New York: Oxford University Press; 2009. Regions and subregions of the cingulate cortex; p. 1-30.
39. Selig J, Preacher K. Monte Carlo method for assessing mediation: An interactive tool for creating confidence intervals for indirect effects. [Internet]. 2008 Available from: <http://quantpsy.org/>.
40. Storch EA, Roberti JW, Roth DA. Factor structure, concurrent validity, and internal consistency of the Beck Depression Inventory-Second Edition in a sample of college students. *Depress Anxiety.* 2004; 19:187–189. [PubMed: 15129421]
41. Spielberger, CD.; Gorsuch, RL.; Lushene, PR.; Vagg, PR.; Jacobs, AG. Manual for the State-Trait Anxiety Inventory (Form Y). Consulting Psychologists Press; 1983.
42. Kumar A, Gupta R, Thomas A, Ajilore O, Helleman G. Focal subcortical biophysical abnormalities in patients diagnosed with type 2 diabetes and depression. *Arch Gen Psychiatry.* 2009; 66:324–330. [PubMed: 19255382]
43. Kumar A, Haroon E, Darwin C, Pham D, Ajilore O, Rodriguez G, Mintz J. Gray matter prefrontal changes in type 2 diabetes detected using MRI. *J Magn Reson Imaging.* 2008; 27:14–19. [PubMed: 18050330]
44. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci.* 2011; 15:37–46. [PubMed: 21109477]
45. Naqvi NH, Rudrauf D, Damasio H, Bechara A. Damage to the Insula Disrupts Addiction to Cigarette Smoking. *Science.* 2007; 315:531–534. [PubMed: 17255515]

46. Stice E, Yokum S, Burger KS, Epstein LH, Small DM. Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. *J Neurosci*. 2011; 31:4360–4366. [PubMed: 21430137]
47. Stice E, Spoor S, Ng J, Zald DH. Relation of obesity to consummatory and anticipatory food reward. *Physiol Behav*. 2009; 97:551–560. [PubMed: 19328819]
48. Kullmann, S.; Heni, M.; Veit, R.; Ketterer, C.; Schick, F.; Häring, HU.; Fritsche, A.; Preissl, H. The obese brain: Association of body mass index and insulin sensitivity with resting state network functional connectivity. *Hum Brain Mapp*. 2011. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21520345>.
49. Qiuhua, Shen; Bergquist-Beringer, S.; Sousa, VD. Major depressive disorder and insulin resistance in nondiabetic young adults in the United States: the National Health and Nutrition Examination Survey, 1999–2002. *Biol Res Nurs*. 2011; 13:175–181. [PubMed: 21044969]
50. Pearson S, Schmidt M, Patton G, Dwyer T, Blizzard L, Otahal P, Venn A. Depression and insulin resistance: cross-sectional associations in young adults. *Diabetes Care*. 2010; 33:1128–1133. [PubMed: 20185745]
51. MacKinnon D, Fairchild A. Current Directions in Mediation Analysis. *Curr Dir Psychol Sci*. 2009; 18:16–22. [PubMed: 20157637]
52. Shrout PE, Bolger N. Mediation in experimental and nonexperimental studies: new procedures and recommendations. *Psychol Methods*. 2002; 7:422–445. [PubMed: 12530702]
53. Friston K. Causal Modelling and Brain Connectivity in Functional Magnetic Resonance Imaging. *PLoS Biol*. 2009; 7:e33. [PubMed: 19226186]
54. Neumeister A, Nugent AC, Waldeck T, Geraci M, Schwarz M, Bonne O, Bain EE, Luckenbaugh DA, Herscovitch P, Charney DS, Drevets WC. Neural and Behavioral Responses to Tryptophan Depletion in Unmedicated Patients With Remitted Major Depressive Disorder and Controls. *Arch Gen Psychiatry*. 2004; 61:765–773. [PubMed: 15289275]
55. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R. Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction. *Curr Top Behav Neurosci*. 2011 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22016109>.

Figure 1.

Anterior mid-cingulate cortex (aMCC) connectivity with the ventral striatum (VS) mediated the relationship between insulin resistance (IR) and depressed mood, as determined by the BDI-II. A cluster in the left aMCC shows stronger connectivity with the left VS in individuals with higher IR. Individuals with higher HOMA-IR values also exhibited stronger connectivity between the left VS and insula, but this connectivity was not related to depressed mood.

A

B

Figure 2.

(A) Connectivity between left ventral striatum (VS) and left anterior mid-cingulate cortex (aMCC) mediated the relationship between HOMA-IR values and BDI-II scores. (B) Connectivity between the left VS and aMCC (adjusted for sex, age, LDL, HDL, BMI and triglyceride levels) predicted log-transformed HOMA-IR values and BDI-II scores.

Table 1

Participant characteristics.

<i>(n = 90)</i>	
Variable	Value
Age (years)	40.4 (6.4)
Gender	46 men (51%) / 44 women (49%)
Body Mass Index (kg/m ²)	28.33 (5.1)
Waist Circumference (inches)	36.91 (5.1)
Cholesterol	
HDL (mg/dL)	47.34 (16.5)
LDL (mg/dL)	119.4 (30.9)
Triglycerides (mg/dL)	105.2 (65.4)
Insulin (μU/mL)	8.88 (7.2)
Glucose (mg/dL)	88.6 (15.0)
Beck Depression Inventory-II	3.98 [*] (4.0)
Cognitive/Affect	3.0 (3.2)
Somatic	1.1 (1.8)
Spielberger Trait Anxiety	33.84 [*] (7.8)

^{*} These scores correspond to sub-threshold levels of depression and anxiety. Data are presented as mean ($\pm$ SD) unless otherwise noted.