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Reduced hippocampal N-acetyl-aspartate (NAA) as a biomarker for overweight ☆

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ABSTRACT

Objective: We previously demonstrated an inverse relationship between both dentate gyrus neurogenesis – a form of neuroplasticity – and expression of the antiapoptotic gene marker, BCL-2 and adult macaque body weight. We therefore explored whether a similar inverse correlation existed in humans between body mass index (BMI) and hippocampal N-acetyl-aspartate (NAA), a marker of neuronal integrity and putatively, neuroplasticity. We also studied the relationship of a potentially neurotoxic process, worry, to hippocampal NAA in patients with generalized anxiety disorder (GAD) and control subjects (CS).

Methods: We combined two previously studied cohorts of GAD and control subjects. Using proton magnetic resonance spectroscopy imaging (^{1}H MRSI) in medication-free patients with GAD ($n = 29$) and a matched healthy control group ($n = 22$), we determined hippocampal concentrations of (1) NAA (2) choline containing compounds (CHO), and (3) Creatine + phosphocreatine (CR). Data were combined from 1.5 T and 3 T scans by converting values from each cohort to z-scores. Overweight and GAD diagnosis were used as categorical variables while the Penn State Worry Questionnaire (PSWQ) and Anxiety Sensitivity Index (ASI) were used as dependent variables.

Results: Overweight subjects (BMI ≥ 25) exhibited lower NAA levels in the hippocampus than normal-weight subjects (BMI < 25) (partial Eta-squared = 0.14) controlling for age, sex and psychiatric diagnosis, and the effect was significant for the right hippocampus in both GAD patients and control subjects. An inverse linear correlation was noted in all subjects between right hippocampal NAA and BMI. High scores on the PSWQ predicted low hippocampal NAA and CR. Both BMI and worry were independent inverse predictors of hippocampal NAA.

Conclusion: Overweight was associated with reduced NAA concentrations in the hippocampus with a strong effect size. Future mechanistic studies are warranted.

1. Introduction

Whereas the hippocampus is commonly associated with memory and learning, a critical role in emotional control and mood and anxiety disorders has also been noted (Apfel et al., 2011). An important but less recognized role for the hippocampus is in the control of food intake and energy balance. For instance, amnesic humans with brain damage that includes the hippocampus have been reported to exhibit insensitivity to signals of hunger and satiety (Hebben et al., 1985; Rozin, 1998), an effect that has also been observed in rats with highly selective lesions that are confined to the hippocampus (Davidson and Jarrard, 1993). Also, obese and post-obese patients tasting a liquid meal showed a decreased activity on positron emission tomography in the posterior hippocampus compared to lean control subjects (DelParigi et al., 2004). Collectively, these results suggest that hippocampal damage might interfere with appetite.

The hippocampus may also play a critical role in the brain's ability to regulate body weight through learning processes (Benoit et al., 2010). Investigators (Davidson et al., 2007) hypothesize that "hippocampal-dependent learning and memory mechanisms translate neurohormonal

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signals of energy balance into adaptive behavioral outcomes involved with the inhibition of food intake". Conversely, the same group proposes the hypothesis "that excessive caloric intake and obesity may be produced by dietary and other factors that are known to alter hippocampal functioning." Reduced hippocampal function and plasticity is observed in rats maintained on diets high in fat and sugar (Kanoski et al., 2007; Liu et al., 2004; Molteni et al., 2002; Monteggia et al., 2004; Wu et al., 2003; Yamada and Nabeshima, 2003). Impaired hippocampal neurogenesis occurs in male rats fed on high-fat diet for 4 weeks (Lindqvist et al., 2006). By contrast, Walker et al. demonstrated that both neonatal leptin treatment and exposure to high-fat diet during the perinatal period increase neurogenesis and neuronal survival in the hippocampal dentate gyrus of young animals, an effect attributed to a reduction of apoptotic processes (Walker et al., 2008).

More recently our own studies found an inverse relationship between body mass in non-obese male nonhuman primates and dentate gyrus doublecortin (reflective of immature neurons) and dentate gyrus expression of BCL-2, an anti-apoptotic gene product (Perera et al., 2011). Ki-67, a marker for precursor proliferative cells, did not correlate with body mass, suggesting that correlations with body mass were maturational rather than proliferation related. Our non-diabetic animals were fed standard monkey chow with occasional fruit treats excluding the potential confound of a high lipid diet causing hippocampal dysfunction. We found markedly high correlations between fasting blood sugar and dentate gyrus BCL-2 ($r = 0.99$) and doublecortin ($r = 0.99$), supporting the premise that one form of dentate gyrus neuroplasticity was involved in metabolic control (Perera et al., 2011).

In light of the overweight/obesity epidemic in the United States (King, 2013; Mitchell et al., 2011), we wished to extend our preclinical studies into the clinical realm, using a non-invasive neuroimaging modality. Proton magnetic resonance spectroscopic imaging (^{1}H MRSI) is well-suited to examine regional alterations in tissue concentrations of neurochemicals that are indicative of brain metabolism (Coplan et al., 2006; Lyoo and Renshaw, 2002). We recently proposed that N-acetylaspartate (NAA), an accepted marker of neuronal integrity, serves as a putative marker of neuroplasticity in GAD (Abdallah et al., 2013), positively tracking hippocampal volume alterations in response to the antiglutamatergic agent, riluzole, in the treatment of GAD.

Since we had observed that neurogenesis rates vary inversely with body mass in nonhuman primates, and our recent studies suggest that NAA may track neurotrophic processes in the hippocampus, we hypothesized that, to the extent neurogenesis is representative of neurotrophic processes in the hippocampus in general, relative elevations in BMI would predict relative reductions in hippocampal NAA levels. However, other possibilities besides the neurogenesis changes restricted solely to the dentate gyrus may be relevant in determining hippocampal NAA, such as synaptic changes, dendritic remodeling or glial cell changes. Although prefrontal cortical volume had previously been associated with reduction in glial cell number (Rajkowska, 2000), recent work by the same group (Cobb et al., 2013) did not demonstrate reductions in glial cell number associated with reduced hippocampal volume in major depressive disorder. Thus the neuropathology associated with lower NAA may have causes other than reduced neurogenesis, which remain to be determined.

We also focus on choline containing compounds (CHO), in part reflective of membrane turnover, which we have shown to be reduced in the centrum semiovale (CSO) of patients with GAD versus healthy volunteers (HV) (Coplan et al., 2006). We examine concentrations of the metabolites of Creatine + phosphocreatine (CR), a potential index of brain metabolism, which we have shown also to be reduced in the CSO of patients with GAD versus HV (Coplan et al., 2006). One study by Massana et al. reported reduced CR in the right medial temporal lobe in patients with panic disorder (Massana et al., 2002). In this study, we wished to examine in a substantial number of subjects combining two cohorts, whether the findings of the Massana et al. study were specific to panic disorder or would be evident in other anxiety disorders. We also examined if parametric effects were evident between CR and measures of worry as measured by the PSWQ.

Thus, the primary aim of our study was to test the hypothesis in humans that an inverse relationship existed between BMI and a marker of hippocampal neuronal integrity, reflected by NAA, on proton magnetic resonance spectroscopy. We might then detect a central biomarker of overweight, facilitating understanding and treatment. Secondarily, we sought to examine the influence, if any, of the diagnosis of GAD, or if not, of worry itself, on the hypothesized relationship between BMI and NAA. Should a relationship between NAA and BMI be observed, it may pave the way for improving our understanding of the hippocampal contribution to the pathophysiology of overweight and the metabolic syndrome.

2. Methods

2.1. Subjects

^{1}H MRSI data were obtained from two previous studies (Mathew et al., 2008, 2009) for a total of 51 subjects—32 women and 19 men, 19 in the former study and 32 in the latter study. 22 control subjects (eight men, fourteen women; mean age ± SD, 33.7 years ± 10.4) and 29 medication-free GAD patients (eleven men, eighteen women; mean age ± SD, 35.1 years ± 11.9) were recruited by advertising or clinician referral.

All patients met the DSM-IV-TR Criteria for GAD as established by the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1995). None of the GAD patients had been taking any psychotropic medication for at least 2 weeks before the MRSI scan. GAD patients had at least moderate worry severity (mean baseline Penn State Worry Questionnaire [PSWQ] (Meyer et al., 1990) score: 64.9 ± 8.1) and severe anxiety sensitivity (Keller, 2002) (mean baseline Anxiety Sensitivity Index [ASI] score: 32.2 ± 11.5). Comorbid diagnoses, determined by SCID, included panic disorder ($n = 7$), social anxiety disorder ($n = 7$), dysthymia ($n = 6$), specific phobia ($n = 4$), depressive disorder not otherwise specified ($n = 2$), adjustment disorder with mixed depressed and anxious mood ($n = 1$), and Bipolar II disorder; most recent depressed in partial remission ($n = 1$).

Exclusion Criteria for GAD patients included the following: major depressive episode or substance abuse/dependence within 6 months of study entry; lifetime histories of psychosis, bipolar disorder, obsessive–compulsive disorder (OCD), eating disorder, or posttraumatic stress disorder (PTSD); or significant medical or neurologic conditions requiring daily medication treatment. In addition, subjects who were pregnant or who had any condition precluding clinical magnetic resonance examination (e.g. pacemaker, metallic prosthesis) were excluded.

Control subjects did not have any current medical conditions or any lifetime history of Axis I psychiatric disorders, according to the SCID-NP interview (Spitzer, 1996). All participants had unremarkable screening laboratory evaluations, including urine toxicology. Written informed consent was obtained and all study procedures were approved by the Institutional Review Board.

2.2. ^{1}H MRSI data acquisition protocol

Neuroimaging studies were conducted on a 1.5-T GE Horizon 5.x Signa MR system in one study (Mathew et al., 2008), and on a 3.0 T GE MRI system using a standard quadrature head coil, in the other study (Mathew et al., 2009). Voxels that best covered the primary regions of interest "ROIs" (right and left hippocampi) in each subject were selected on the basis of their location on the matching high-resolution MR localizer images.

Following sagittal scout images, a four-section T1-weighted axial/oblique localizer imaging series, angulated parallel to the Sylvian fissure (Fig. 1A), was acquired, with a slice thickness of 15 mm and an interslice