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Age-Related Increases in Basal Ganglia Glutamate are Associated with TNF-Alpha, Reduced Motivation and Decreased Psychomotor Speed During IFN-alpha Treatment: Preliminary Findings

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Abstract

Inflammation-induced alterations in central nervous system (CNS) metabolism have focused on glutamate. At excessive concentrations, glutamate is toxic to glia and neurons, and inflammatory cytokines have been shown to influence glutamate metabolism by blocking glutamate reuptake and increasing glutamate release. Increased glutamate has also been found in depression, a disorder associated with increased inflammation. Data by our group have shown increased glutamate as measured by magnetic resonance spectroscopy (MRS) in basal ganglia and dorsal anterior cingulate cortex of patients administered the inflammatory cytokine interferon (IFN)-alpha. Given data that increasing age is associated with an exaggerated CNS inflammatory response, we examined whether older age (>55 years) would be associated with a greater IFN-alpha-induced increase in CNS glutamate. Using a longitudinal design, 31 patients with hepatitis C virus (HCV) underwent MRS, blood sampling for inflammatory markers, and behavioral assessments before (Visit1) and after four weeks (Visit 2) of either IFN-alpha (n=17) or no treatment (n=14). Older patients treated with IFN-alpha exhibited a significantly increased glutamate from Visit 1 to Visit 2 as reflected by the glutamate/creatinine ratio (Glu/Cr) in left basal ganglia compared to older controls and younger IFN-alpha-treated and untreated subjects. In

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addition, increased Glu/Cr in older but not younger IFN-alpha-treated and untreated patients was associated with increased tumor necrosis factor, reduced motivation as measured by the Multidimensional Fatigue Inventory and increased choice movement time on the Cambridge Neuropsychological Test Automated Battery. Taken together, these preliminary data support the notion that older age may interact with inflammation to exaggerate the effects of inflammatory stimuli on CNS glutamate and behavior.

Keywords

Aging; Inflammation; Glutamate; Basal Ganglia; Motivation; Cytokine; TNF; IFN-alpha; Psychomotor Speed

1. Introduction

Patients exposed to the inflammatory cytokine interferon (IFN)-alpha were found to exhibit increased glutamate in left basal ganglia and dorsal anterior cingulate cortex (dACC) as determined by single voxel magnetic resonance spectroscopy (MRS)(Haroon et al., 2014). Increased glutamate was in turn correlated with depressive symptoms. These data are consistent with increased CNS glutamate in bipolar and some unipolar depression (Yuksel and Ongur, 2010), both of which exhibit increased inflammatory markers (Miller et al., 2009).

Regarding mechanisms by which inflammation may increase CNS glutamate, inflammatory cytokines can decrease expression of glutamate transporters on astrocytes, resulting in decreased glutamate reuptake (Tilleux and Hermans, 2007), and stimulate astrocytic glutamate release (Malarkey and Parpura, 2008). Moreover, inflammatory cytokines can activate indoleamine 2,3 dioxygenase (IDO), an enzyme which catabolizes tryphophan into kynurenine that in turn can be converted into quinolinic acid (Miller et al., 2009). Quinolinic acid is an agonist at glutamate receptors and can also stimulate glutamate release while inhibiting astrocytic glutamate reuptake (Miller et al., 2009; Savitz et al., 2014; Tavares et al., 2002). At high concentrations, extracellular glutamate is toxic to both neurons and glia. However, it should be noted that current MRS methodology is limited in discriminating extra- versus intra-cellular glutamate, making it difficult to determine whether increases in CNS glutamate as determined by MRS represent increased extracellular glutamate concentrations or increased intracellular pools (Haroon et al., 2014).

One process that may interact with depression and the impact of inflammatory stimuli on CNS glutamate is aging. Advancing age is associated with chronic, low-grade central and peripheral inflammatory responses (Franceschi and Campisi, 2014; Norden and Godbout, 2013). As a consequence of aging, microglia have been shown in laboratory animals to exhibit a “primed” phenotype that is associated with an exaggerated and prolonged inflammatory response that correlates with increased depressive-like behavior and cognitive deficits (Norden and Godbout, 2013; Salminen et al., 2011). In addition, aging is associated with increased inflammation-induced CNS IDO activity in mice (Godbout et al., 2008). Moreover, expression of glutamate transporters appears to decline with age (Sheldon and Robinson, 2007). Finally, as indicated above, CNS glutamate is abnormally regulated in

patients with mood disorders including those with late-life depression (Binesh et al., 2004). These data suggest that the intersection of aging, inflammation and depression in older patients may represent a “perfect storm” of glutamate pathology.

We therefore explored effects of aging on the glutamate response to IFN- α in patients with hepatitis C virus (HCV) and determined whether these effects were associated with alterations in inflammatory markers and behaviors previously shown to be altered in IFN- α -treated patients including tumor necrosis factor (TNF) and its soluble receptor sTNFR2, motivation, and motor activity (Capuron et al., 2012; Majer et al., 2008; Raison et al., 2010).

2. Materials and Methods

2.1 Sample

Patients were recruited from the Emory University Division of Digestive Diseases and were serum positive for HCV-RNA. IFN- α treatment was determined by patient consultation with their gastroenterologist independent of study participation. Patients were excluded for advanced liver disease or liver disease due to any etiology other than HCV. Other exclusion criteria included unstable cardiovascular, endocrinologic, hematologic, renal or neurologic disease (based on medical history, physical exam and laboratory testing); a lifetime diagnosis of schizophrenia or bipolar disorder or current major depression based on DSM IV criteria determined by Structured Clinical Interview for DSM IV (SCID)(First et al., 2002); and substance abuse or dependence within the past year. Lifetime diagnosis of major depression was not exclusionary; however patients were required to be antidepressant-free for at least 1 year and off all other psychotropic medications for at least 1 month before participation. One patient was included after taking a sedative/hypnotic the night before scanning. Patients were free of contraindications to MRI including metallic implants and claustrophobia. The median age of the sample (55 years) was used to divide IFN- α -treated and untreated subjects into “older” (age>55 years) and “younger” (<55 years) subgroups. The study represents a secondary analysis of patients described elsewhere (Haroon et al., 2014).

2.2 Study Design

A longitudinal, repeated measures design was employed. Individuals were administered MRS scans, behavioral ratings, neurocognitive testing, and blood sampling on the same day within 1 week before IFN- α treatment (Visit 1) and within 4–6 days following the 4th IFN- α dose (four doses given at weekly intervals)(Visit 2). The untreated control group underwent similar assessments at Visit 1 (baseline) and Visit 2 (~4 weeks later). The mean duration between Visit 1 and Visit 2 did not differ between groups (IFN- α : 37.1 $\pm$ 12.0 days versus controls: 31.6 $\pm$ 6.0 days)(F[3,27]=2.24, p=0.11). IFN- α was administered as either pegylated IFN- α -2b (Pegintron, Merck 1.5 mcg/kg/week SC)(n=1) or pegylated IFN- α -2a (Pegasys, Roche 184 mcg/week/kg SC)(n=16) plus ribavirin (800–1200 mg/d) (n=17) in the presence or absence of telaprevir (2250 mg/d TID)(n=10) or boceprevir (2400 mg/d)(n=1) as determined by the treating physicians. All patients were asked to refrain from caffeine and tobacco the day of the study. Study procedures were approved *a priori* by

Emory University Institutional Review Board, and all subjects provided written informed consent.

2.3 Behavioral Assessments

Depressive symptoms were evaluated by the 17-item Hamilton Depression Rating Scale (Hamilton, 1960) and the Multidimensional Fatigue Inventory (MFI)(Smets et al., 1995). The MFI assesses five dimensions of fatigue commonly found among medically ill patients including general fatigue, mental fatigue, physical fatigue, reduced activity, and reduced motivation. Psychomotor speed was assessed using the reaction time task of the Cambridge Neuropsychological Test Automated Battery (CANTAB)(Majer et al., 2008). The test includes simple and five-choice segments that provide distinction between reaction time (simple and choice reaction time) and movement time (simple and choice movement time) (Majer et al., 2008). Reaction time is the speed with which the subject releases the press pad in response to the onset of a stimulus. Movement time (motor speed) is the time taken to touch the stimulus on the computer screen after the press pad had been released (Majer et al., 2008).

2.4 Inflammatory Markers

Plasma was isolated from centrifuged whole blood collected in EDTA tubes at the same time of day (~2pm) to reduce circadian variations. Samples were stored at -80°C for batched assay. Plasma TNF and sTNFR2 were measured in duplicate by quantitative enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN). Inter- and intra-assay variability were reliably less than 12% for TNF-alpha and 10% for sTNFR2. Lower limits of detection for TNF and sTNFR2 were 0.19pg/ml and 2.3 pg/ml, respectively. Research staff was blinded to clinical status of subjects, and no samples were below detection limits.

2.5 MRI Acquisition

In vivo levels of glutamate as reflected by the glutamate (Glu) to creatine (Cr) ratio (Glu/Cr) were obtained by ^1H MRS from 2 voxels each of $17 \times 30 \times 17 \text{ mm}^3$ located on the right and left basal ganglia and one voxel sized $20 \times 30 \times 10 \text{ mm}^3$ located on the dACC (BA24). Imaging was performed on a Siemens 3T MR system as described (Haroon et al 2014). In brief, a standard point-resolved spectroscopy sequence (PRESS) was used for spatial localization, and chemical shift selective (CHESS) pulses were used for water suppression. The parameters used for spectral acquisition were TR=3000msec, TE=30msec, sampling size=1024, 128 averages. Post processing was accomplished using LC Model software. Only Glu/Cr values whose metabolite variance ratios (Cramer-Rao lower bounds-CRLB) were <20% were used for analysis. MRS data from 2 left basal ganglia and 1 dACC voxels from older IFN-alpha treated individuals were excluded due to CRLB>20%. Water was used as the internal reference, and creatine (Cr) was used to scale the data to model spectra across subjects (per LC Model Manual) and to minimize intra- and inter-individual variation (Provencher, 2012). No significant between group difference in Cr concentrations was seen in any voxels, nor were there any differences in Cr concentrations between Visit 1 and Visit 2 for either group.

2.6 Statistical Analysis

One-way analysis of variance (ANOVA)(or Fisher's exact test where appropriate) was used to compare sociodemographic and clinical variables among older and younger IFN-alpha-treated and control subjects at baseline. Repeated measures ANOVA was used to assess the primary outcome of this study which was the effect of age (older versus younger), treatment (IFN-alpha versus control) and visit (Visit 1 versus Visit 2) and their interaction on the indicated biological and behavioral variables (Table 2). Post hoc comparisons of specific means of interest were performed using t tests. In brain regions where significant age by treatment by visit interactions were found, linear regression analyses were used to determine correlations between delta values calculated by subtracting Visit 1 from Visit 2. Using this strategy, the relationship between delta CNS glutamate and 1) inflammatory markers, 2) behavioral measures and 3) assessments of psychomotor speed were evaluated in older and younger subjects in independent analyses. Sex, body mass index (BMI) and educational status were included in all statistical models. Non-normally distributed variables (Glu/Cr, TNF-alpha and sTNFR2) were log-transformed for the regression analyses. Raw values are provided in Table 2. Statistical computations were performed using IBM-SPSS (International Business Machines, New York, NY). Power calculations were performed using G*Power (Faul et al., 2007).

3. Results

3.1 Sample

Thirty-one subjects (17 IFN-alpha-treated patients and 14 HCV controls) met inclusion and exclusion criteria and were enrolled (Table 1). The younger IFN-alpha-treated group exhibited a trend toward a higher BMI than the other three groups ($p=0.06$). There was also a significant main effect of age on education ($F(3,27)=5.8$, $p<0.003$) with the two older groups exhibiting higher education levels than the two younger groups (all $p<0.05$). These variables (BMI and education) along with sex were therefore included in all statistical models.

3.2 Glutamate metabolites

As we have previously reported, there was a significant treatment by visit interaction for both the left basal ganglia and the dACC ($F[1,22]=4.76$, $p=0.04$, $d=0.48$, power=0.68 and $F[1,23]=9.68$, $p=0.005$, $d=0.65$, power=0.94, respectively)(Haroon et al., 2014) with IFN-alpha-treated subjects exhibiting significantly greater increases from Visit 1 to Visit 2 in the Glu/Cr ratio in the left basal ganglia and dACC ($p<0.05$). Regarding the focus of the current study on age, no significant main effects of age or visit were found for any of the brain regions examined (all $p>0.40$). Nevertheless, there was a significant age by treatment by visit interaction for the left basal ganglia ($F[1,22]=10.4$, $p=0.004$, $d=0.68$, power=0.96) with older IFN-alpha-treated patients exhibiting significantly greater increases in left basal Glu/Cr from Visit 1 to Visit 2 than older controls and both younger IFN-alpha-treated and control individuals (all $p<0.05$)(Table 2). No age by treatment by visit interactions were found for the right basal ganglia or the dACC.

3.3 Main Effects, Associations and Interactions Involving Left Basal Ganglia Glutamate, Inflammatory Markers, and Behavioral and Cognitive Measures

There was a significant treatment by visit interaction for TNF and sTNFR2 ($F[1,25]=7.7$, $p<0.02$, $d=0.56$, power=0.83 and $F[1,24]=13.8$, $p=0.001$, $d=0.75$, power=0.99, respectively) with IFN-alpha-treated patients exhibiting significantly greater increases in plasma concentrations of TNF and sTNFR2 from Visit 1 to Visit 2 than controls (Table 2). There was also a significant interaction between age and visit for sTNFR2 ($F[1,24]=6.6$, $p=0.02$, $d=0.53$, power=0.76), with older patients exhibiting significantly higher plasma concentrations of sTNFR2 than younger patients. No treatment by age by visit interactions for TNF-alpha or sTNFR2 were found. Regarding reduced motivation and choice movement time, a significant age by treatment by visit interaction was found for both variables ($F[1,24]=4.67$, $p=0.04$, $d=0.44$, power=0.58 and $F[1,18]=6.9$, $p=0.02$, $d=0.62$, power=0.91, respectively) with older IFN-alpha-treated patients exhibiting significantly greater increases in reduced motivation from Visit 1 to Visit 2 than older controls and both younger IFN-alpha-treated and control individuals (all $p<0.05$)(Table 2).

As shown in Figure 1A, delta (Visit2-Visit1) log left basal ganglia glutamate was higher in older IFN-alpha-treated patients than all other groups (all $p<0.05$). Linear regression analyses of the relationship between delta log left basal ganglia Glu/Cr and delta log inflammatory markers (log TNF and log sTNFR2) revealed a significant association between delta log TNF and delta log left basal ganglia Glu/Cr in older IFN-alpha-treated and control patients with sex, BMI and education included in the model ($\beta=0.61$, $p=0.026$) (Figure 1B). A significant association was also found between delta log left basal ganglia Glu/Cr and delta reduced motivation in older IFN-alpha-treated and control subjects including other MFI-subscores and the HAM-D-17 in the model as well as sex, BMI and education ($\beta=0.66$, $p=0.014$)(Figure 1C). Finally, delta log left basal ganglia Glu/Cr was significantly associated with increased delta choice movement time in older IFN-alpha-treated and control subjects including delta values of other reaction and movement time measures as well as sex, BMI and education in the model ($\beta=0.56$, $p=0.027$)(Figure 1D). Of note, similar associations as noted above were found in older IFN-alpha-treated subjects alone (delta log TNF-: $r=0.62$; delta reduced motivation: $r=0.47$ and delta choice movement time: $r=0.97$) controlling for sex, BMI and education. Nevertheless, due to the small sample size, these correlations did not reach statistical significance (all $p>0.15$). Correlations in older control patients were either in the opposite direction (log TNF-alpha: $r=-0.67$) or lower than in older IFN-alpha-treated patients (delta reduced motivation: $r=0.33$ and delta choice movement time: $r=0.25$, both $p>0.34$). No significant associations between the indicated delta variables and delta Glu/Cr were found in younger IFN-alpha-treated and control subjects using linear regression analyses or partial correlations.

3.4 Other MRS metabolites

An analysis of the spectral peaks of other brain metabolites showed a significant treatment by visit interaction for choline (Cho/Cr) in the dACC ($F[1,24]=4.63$, $p=0.04$, $d=0.44$, power=0.57)(see Supplementary Data) with older and younger IFN-alpha-treated subjects exhibiting significantly decreased choline from Visit 1 to Visit 2. No main effects or interactions were found for the other MRS metabolites.

4. Discussion

In the current study, older individuals (age>55 years) were found to exhibit significantly greater increases in left basal ganglia Glu/Cr following IFN-alpha administration than older controls and younger IFN-alpha-treated and control individuals. In addition, there was a significant relationship between left basal ganglia Glu/Cr and TNF, reduced motivation and increased movement time in older but not younger individuals. These data are consistent with previous work by our group demonstrating that IFN-alpha increased glutamate in left basal ganglia (Haroon et al., 2014), as well as data that IFN-alpha is associated with reduced motivation and increased choice movement time (Capuron et al., 2012; Majer et al., 2008). Moreover, these data are consistent with previous work demonstrating a relationship between TNF and behavioral changes in IFN-alpha-treated subjects (Raison et al., 2010). Importantly however, these data extend our prior findings by suggesting that increased age may significantly interact with inflammation to exaggerate the effects of IFN-alpha on basal ganglia glutamate while also revealing its association with TNF (not seen in the group as a whole or in younger subjects) and increasing its impact on motivation and motor activity.

As noted previously, aging may be associated with a more vigorous CNS response to inflammatory stimuli such as IFN-alpha. Indeed, aging astrocytes have been found to exhibit a morphologically distinct 'senescence-associated secretory phenotype' (SASP) (Salminen et al., 2011). The transition from resting to a SASP state is believed to result in increased secretion of cytokines under basal and activated conditions (Norden and Godbout, 2013; Salminen et al., 2011). Similar age-associated changes have been described in microglia, which become primed for activation and are resistant to regulation (Norden and Godbout, 2013). Furthermore, evidence suggests that invading macrophages which gain access to the brain during inflammatory states (e.g. during IFN-alpha administration) and increased permeability of the blood-brain-barrier (e.g. as a function of aging), may further contribute to increased glutamate (Piani et al., 1991). While the degree of SASP-transformation as a function of age varies according to tissue, preliminary evidence suggests that glial elements in basal ganglia show higher concentrations of SASP markers (Campuzano et al., 2009). Thus it is possible that higher concentrations of SASP astrocytes, primed microglia and/or invading macrophages in basal ganglia might predispose to a greater impact of inflammatory stimuli preferentially in this brain region with resultant consequences on relevant biochemical and behavioral outcomes in older individuals. Regarding the laterality of the findings, several studies have supported a relationship between left basal ganglia lesions and behavioral changes including depression following stroke and traumatic brain injury (Haroon et al., 2014). Nevertheless, there is limited data to suggest the underlying immunological mechanisms involved.

While the study has several strengths such as a longitudinal design; a standardized immune stimulus; parallel pre-post measurements of CNS glutamate, inflammatory markers and behavior in medication-naïve individuals, there are limitations which warrant consideration. First, the number of individuals in the different subgroups was small, and therefore the findings should be considered preliminary. Second, our division of groups into "older" and "younger" based on a median split (of age 55) should be considered arbitrary, and the design

precluded recruitment of individuals older than 65 years of age, who may show even greater aging effects.

In summary, the findings from this preliminary study are consistent with previous results indicating that older individuals exhibit an exaggerated CNS response to inflammatory stimuli in brain regions that may exhibit an overrepresentation of primed glial cells and thereby a multiplied risk of inflammation-induced changes including increased CNS glutamate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Older age and IFN-alpha interacted to increase left basal ganglia glutamate
- Increased TNF in older subjects correlated with increased basal ganglia glutamate
- Increased basal ganglia glutamate correlated with reduced motivation in older subjects
- Increased basal ganglia glutamate correlated with motor slowing in older subjects

Figure 1. Increased Left Basal Ganglia Glutamate in Older IFN-alpha-Treated Subjects and Correlation of Left Basal Ganglia Glutamate with TNF, Reduced Motivation and Motor Slowing in Older Hepatitis C Subjects

A. Delta (Visit2-Visit1) log left basal ganglia glutamate (Glu/Cr) is higher in older (>55 years) interferon (IFN)-treated subjects than older controls and younger (<55 years) IFN-alpha-treated and untreated subjects. **B.** Increased delta log TNF correlates with increased delta log left basal ganglia Glu/Cr in older IFN-alpha-treated and control subjects ($B=0.61$, $p=0.026$). **C.** Increased delta log left basal Glu/Cr correlates with increased delta reduced motivation in older IFN-alpha-treated and untreated subjects ($B=0.67$, $p=0.014$). **D.** Increased delta log left basal ganglia Glu/Cr correlates with increased delta choice movement time in older IFN-alpha-treated and untreated subjects ($B=0.61$, $p=0.04$). Of note, one subject did not complete the CANTAB reaction time task. *- $p<0.05$, ***- $p<0.001$; Open circles: IFN-alpha; Closed circles: controls

Table 1

Sociodemographic Characteristics of the Study Sample

Variable	Older >55 years		Younger <55 years		P
	IFN-alpha (n=8)	Control (n=7)	IFN-alpha (n=9)	Control (n=7)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Age	59.9 (3.1)	58.4 (2.4)	49.3 (8.9)	52.7 (2.1)	0.001
Sex Females (%)	3 (37.5%)	1 (14.3%)	5 (55.6%)	2 (28.6%)	0.70
BMI	28.0 (3.8)	28.1 (2.4)	32.6 (4.8) [‡]	27.1 (3.7)	0.03
Education (years)	16.2 (3.3) [*]	15.4 (3.2) [*]	11.7 (2.0)	12.9 (1.1)	0.003
Employed no. (%)	6 (75%)	3 (42.9%)	5 (55.6%)	7 (100%)	0.22
Smoking no. (%)	0 (0%)	3 (42.9%)	2 (22.2%)	1 (14.3%)	0.24
Past history no. (%)	2 (25%)	3 (42.9%)	1 (11.1%)	0 (0%)	0.24
Drug Abuse no. (%)	2 (25%)	3 (42.9%)	3 (33.3%)	1 (14.3%)	0.76

BMI: body mass index; IFN: interferon; no.: number;

^{*} significantly higher than respective younger groups;

[‡] higher than other groups (p=0.06)

Table 2

Mean ± SD CNS Glutamate, Peripheral Inflammatory Markers and Behavior in IFN-alpha-Treated and Control, Older and Younger Patients at Baseline (Visit 1) and 4 Weeks (Visit 2)

Variable	Older >55 years				Younger <55 years			
	IFN-alpha (n=8)		Control (n=7)		IFN-alpha (n=9)		Control (n=7)	
	Visit 1	Visit 2	Delta		Visit 1	Visit 2	Delta	
Left Basal Ganglia Glu/Cr ^{a,c}	0.74 ± 0.15 (n=6)	1.07 ± 0.12	0.33 ± 0.23 *		0.96 ± 0.19 (n=7)	0.85 ± 0.21	-0.12 ± 0.24	
Right Basal Ganglia Glu/Cr	0.90 ± 0.25	0.87 ± 0.16	-0.03 ± 0.24		0.90 ± 0.14	0.89 ± 0.09	-0.01 ± 0.23	
dACC Glu/Cr ^d	1.16 ± 0.12 (n=7)	1.31 ± 0.11	0.15 ± 0.18		1.28 ± 0.17 (n=7)	1.26 ± 0.12	-0.03 ± 0.14	
TNF-alpha (pg/ml) ^d	5.91 ± 7.83	6.72 ± 7.12	0.72 ± 1.22 [†]		3.44 ± 2.59	3.55 ± 2.87	-0.80 ± 0.39	
sTNFR2 (ng/ml) ^{b,c}	5.15 ± 1.65	8.04 ± 4.06	3.36 ± 1.43 [†]		6.02 ± 2.80	7.07 ± 4.32	1.04 ± 2.41	
Reduced motivation ^c	7.63 ± 2.87	11.88 ± 3.87	4.25 ± 3.84 *		10.42 ± 1.99	8.43 ± 2.98	-2.00 ± 3.60	
Choice movement time ^c	536.70 ± 112.22	623.44 ± 128.94	87.10 ± 89.31		612.87 ± 196.57	578.65 ± 156.78	-34.22 ± 78.34	
					481.95 ± 132.17	482.35 ± 148.25	0.40 ± 64.80	
					6.29 ± 2.14	9.33 ± 3.64	-0.22 ± 4.84	
					7.58 ± 2.84	8.04 ± 4.06	1.75 ± 1.84 [†]	
					3.01 ± 1.33	4.16 ± 1.95	1.19 ± 1.56 [†]	
					1.34 ± 0.15 (n=7)	1.44 ± 0.14	0.16 ± 0.17	
					0.90 ± 0.27	0.86 ± 0.18	0.03 ± 0.22	
					0.93 ± 0.28 (n=7)	0.99 ± 0.15	0.04 ± 0.13	
					1.02 ± 0.16	0.95 ± 0.12 (n=9)	0.04 ± 0.13	
					0.91 ± 0.20	0.86 ± 0.18	0.03 ± 0.22	
					0.91 ± 0.20	0.86 ± 0.18	0.03 ± 0.22	
					1.18 ± 0.17	1.44 ± 0.14	0.16 ± 0.17	
					2.64 ± 1.07	4.16 ± 1.95	1.19 ± 1.56 [†]	
					7.33 ± 2.58	8.04 ± 4.06	1.75 ± 1.84 [†]	
					6.29 ± 2.75	9.33 ± 3.64	-0.22 ± 4.84	
					612.87 ± 196.56	482.35 ± 148.25	0.40 ± 64.80	
					45.62 ± 51.21	582.54 ± 112.92	582.54 ± 112.92	

CNS: central nervous system; dACC: dorsal anterior cingulate cortex; Glu/Cr: glutamate/creatinine ratio; IFN: interferon; TNF: tumor necrosis factor; sTNFR2: soluble tumor necrosis factor receptor type 2;

^a significant treatment by visit interaction (p<0.05);

^b significant age by visit interaction (p<0.05);

^c significant treatment by age by visit by time interaction (p<0.05);

^{*} significantly higher than all other groups p<0.05;

[†] significantly higher than respective controls