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Reduced Density of Calbindin Immunoreactive GABAergic Neurons in the Occipital Cortex in Major Depression: Relevance to Neuroimaging Studies

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Abstract

Background—Several lines of evidence suggest dysfunction of the GABAergic system in Major Depressive Disorder (MDD). Neuroimaging studies report reduced levels of GABA in the dorsolateral prefrontal and occipital cortex of depressed patients. Our previous postmortem study revealed a reduction in the density and size of Calbindin-immunoreactive (CB-IR) GABAergic neurons in the prefrontal cortex in MDD. The goal of this study was to test whether the changes in CB-IR neurons can also be detected in the occipital cortex where neuroimaging studies report a prominent GABA decrease.

Methods—A three-dimensional cell counting probe was used to assess the cell packing density and size of CB-IR neurons in layer II of the occipital cortex in 10 MDD subjects, and 10 psychiatrically healthy controls.

Results—The density of CB-IR neurons was significantly decreased by 28% in MDD subjects as compared to the control group. The size of CB-IR neurons was unchanged in MDD subjects when compared to controls.

Conclusions—The reduction in the density of CB-IR GABAergic neurons in the occipital cortex in depression is similar to that observed previously in the prefrontal cortex. Deficit in cortical GABAergic interneurons may contribute to the low GABA levels detected in neuroimaging studies in MDD patients.

Keywords

GABAergic neurons; calbindin immunoreactivity; major depression; postmortem; occipital cortex

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Introduction

There is increasing evidence that γ -aminobutyric acid (GABA) deficits are involved in the pathophysiology of major depressive disorder (MDD), (see 1 for comprehensive review). Neuroimaging studies demonstrate consistent reductions in cortical GABA content in occipital and prefrontal cortex of depressed patients (2,3,4,5,6). Similarly, the low GABA concentrations were observed in cerebrospinal fluid and in plasma in MDD patients (1,7). These clinical observations of reduced GABAergic content associated with MDD are supported by postmortem studies on human brain tissue. Our recent postmortem morphometric analysis shows prominent reductions in the density and size of calbindin-IR (CB-IR) GABAergic neurons in layer II of Brodmann area 9 of the dorsolateral prefrontal cortex (dlPFC) of MDD subjects (8). Moreover, in a follow up study, a significant decrease in the level of glutamic acid decarboxylase (GAD-67), the GABA synthesizing enzyme has been detected in the same cortical region in depressed subjects (9). Other groups also found reductions in GAD-65/67 immunopositive structures in the dlPFC (10) and in GAD protein level in the cerebellum (11) in depression, further supporting GABAergic dysfunction in MDD.

However, none of these human postmortem studies have investigated GABAergic indices in the occipital cortex, the brain region, where prominent GABA decreases were originally reported by neuroimaging studies in living MDD patients. Since the subpopulation of CB-IR GABA neurons and not parvalbumin-IR neurons was selectively reduced in the dlPFC in our cohort of MDD subjects (8), the goal of the present study was to determine whether the subpopulation of CB-IR neurons would also be altered in the occipital cortex in MDD.

Materials And Methods

Human Subjects

Postmortem brain samples were collected at autopsy at the Cuyahoga County Coroner's Office in Cleveland, OH from 20 subjects. Informed written consent was obtained from the legal next-of-kin of all subjects. Next-of-kin were interviewed and retrospective psychiatric assessments were conducted in accordance with Institutional Review Board policies as described previously (12). Ten subjects met clinical criteria for MDD, and the other 10 subjects (termed normal controls) did not meet criteria for an Axis I diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders-Revised DSM-IV (American Psychiatric Association, 1994), (Table 1). A trained interviewer administered either the Schedule for Affective Disorders and Schizophrenia: lifetime version (SADS-L) or the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID) to knowledgeable next-of-kin of all subjects, as previously described (13,14). Diagnoses for Axis I disorders were assessed independently by a clinical psychologist and a psychiatrist, and consensus diagnosis was reached in conference, using all available information from the knowledgeable informants, the coroner's office, and any available previous hospitalizations and doctor's records. Research on the psychological autopsy method has revealed that diagnoses from structured clinical interviews with family members are in good agreement with diagnoses based on reviewing the subject's medical records (15, 16). In addition, strong inter-rater concurrence has been obtained when a structured clinical interview was used to collect information from depressed patients vs. information collected from next-of-kin (17). Of the 10 subjects that met DSM-IV criteria for MDD via retrospective assessment, there was no evidence that four ever sought mental health treatment, another two were seen by primary care physicians, and the remaining four were seen by psychiatrists. Six of the ten subjects received a pre-mortem diagnosis of MDD. Responses from the subjects evaluated with the SADS-L were also recorded in the SCID, and these subjects met DSM-IV criteria for MDD using information collected with either structured diagnostic interview.

Toxicology assays were performed by the coroner's office using gas chromatography with mass selective spectrometry or high performance liquid chromatography to detect the following classes of compounds in blood and urine: antidepressant or antipsychotic drugs, barbiturates, benzodiazepines, sympathomimetic amines, cocaine and its metabolites, opiates, phencyclidine, cannabinoids, and antiepileptic drugs. According to the medical records 5 out of 10 MDD subjects had prescription for antidepressants and 3 out of these 5 subjects had prescription for antidepressants in the last month of life. However, only one MDD subject had detectable levels of an antidepressant medication in postmortem toxicology screening (Table 1). Six of the 10 MDD subjects died by suicide. The control subjects were group matched with the depressed subjects for age, gender, post-mortem interval (PMI), time in formalin (TF), and brain tissue pH (Table 1).

Tissue Preparation

Tissue was collected at autopsy and fixed in phosphate-buffered formalin (10%) as described previously (12). Blocks of tissue from the occipital cortex of each subject were embedded in 12% celloidin. Morphometric parameters were measured within the calcarine sulcus of Brodmann area 17 (the primary visual cortex - V1). Area 17 has been chosen as previously published MRS studies measuring GABA in depressed patients were centered primarily on V1 region.

The tissue blocks were sectioned at 40 μm , and stained for either Nissl substance or immunohistochemistry using antibodies to calbindin-D28K. Nissl-stained sections were used to identify cytoarchitectonic features of area 17 and to draw the borders between individual cortical layers. These laminar borders were then imposed on the adjacent (200 μm apart from Nissl section) immunostained sections to determine the laminar distribution of immunoreactive cells.

Immunocytochemistry

Celloidin-embedded sections were immunostained after the removal of celloidin (18). The sections were incubated with a rabbit polyclonal anti-calbindin-D28K antibody at 1:750 dilution (Millipore/Chemicon, AB1778). Binding of these antibodies was detected with a secondary anti-rabbit antibody according to the ABC method (ABC kit, Vector Laboratories, CA). The immunostained sections were adjacent to or within 200 μm of the Nissl stained sections used for the identification of the relevant area. To minimize the variability in the intensity of staining, sections from depressed and control subjects were stained simultaneously. For each subject, three coronal sections were used for morphometric analyses.

Morphometric Analyses

The estimation of cell packing density and size of CB-IR neurons were carried out by the investigator naive to the diagnoses. CB-IR neurons were analyzed in cortical layer II, as the majority of CB-IR neurons were located in this layer. The density of CB-IR neurons was estimated with a $\times 40$ oil immersion objective (1.0 numerical aperture) using the 'Optical Fractionator' probe of Stereo Investigator software (version 8.21.4 32-bit, MicroBrightField Inc.). In each section 40–60 3-D counting boxes (70 $\times$ 70 $\times$ 14 μm ; 1 μm guard zone from the top) were placed randomly within the contour outlining layer II. The packing density of immunopositive cells was calculated in each section by dividing the total number of cells counted in all boxes by the combined volume of all counting boxes. The size of CB-IR neurons was estimated by measuring the volume of immunoreactive cell bodies with the 'Nucleator' probe of the Stereo Investigator software.

Statistical Analyses

Mean values for cell density and neuronal size (somal volume) obtained from the three sections of each subject were compared between the groups using analysis of covariance (ANCOVA) with age, PMI, brain tissue pH and TF as covariates. Pearson correlation analysis was used to assess the influence of confounding factors such as age, age at onset of depression, PMI, pH, and TF on neuronal density and size (SPSS, version 16.0).

Results

The density of CB-IR neurons was significantly reduced by 28% in the MDD subjects (21.2 ± 2.7 neurons $\times 10^3/\text{mm}^3$) as compared to the age-matched control group (29.5 ± 2.2 neurons $\times 10^3/\text{mm}^3$; ANCOVA, $F(1,14) = 7.58$, $p = 0.016$), Figures 1 and 2. The reduced density in MDD subjects does not appear to be specifically related to death by suicide since there was almost no difference in the mean density of CB-IR neurons between the 6 MDD suicide (20.8 ± 3.0 neurons $\times 10^3/\text{mm}^3$) and 4 MDD non suicide (21.6 ± 5.5 neurons $\times 10^3/\text{mm}^3$) subjects, Figure 3.

The average size of CB-IR cell bodies in MDD group ($481.9 \pm 33.5 \mu\text{m}^3$) was not significantly different from that of controls ($508.5 \pm 28.2 \mu\text{m}^3$; ANCOVA, $F(1,14) = 0.261$, $p = 0.618$), Figure 4.

Correlation analyses revealed no association between the density or size of CB-IR neurons and confounding variables such as: age at the time of death, PMI, brain pH and storage time in formalin, (for statistics, see Table 2). The lack of influence of confounding variables on the CB neurons density or size was observed whether all subjects (MDD + controls) or each of the diagnostic groups were tested separately. Similarly, there was no correlation between the density or size of CB-IR neurons and the age at onset of depression in MDD group, Table 2.

Tobacco use is a potentially important confounding variable in this study since associations between smoking status and abnormalities of GABA system have been reported (19,20), and 6 out of 10 MDD subjects were smokers, while only 1 out 10 controls smoked. However, the influence of smoking did not appear to be major factor accounting for the reduced densities of CB-IR neurons in MDD subjects in this study since the mean densities of CB-IR neurons in MDD smokers and MDD non smokers were 22.28 ± 3.63 and 19.43 ± 4.23 cells $\times 10^3/\text{mm}^3$, respectively.

Discussion

The present study demonstrates marked (28%) reductions in the density of calbindin-IR neurons in layer II of the occipital cortex in MDD subjects as compared to controls. However, the size of CB-IR neurons was not significantly different between the two groups. All (but one) of our MDD subjects were antidepressant free at the time of death as revealed by postmortem toxicology screening, suggesting this was largely a medication-free population. This deficit in GABA neurons is consistent with multiple proton magnetic resonance spectroscopy studies showing prominent reductions in GABA levels in the occipital cortex of antidepressant free, living depressed patients (2,3,5,6). Moreover, a recent neuroimaging study found significant reductions in dorsomedial and dorsolateral prefrontal cortex GABA content in MDD patients, while, GABA levels were unchanged in the frontal polar and ventromedial prefrontal regions in the same patients (4). This is in strong agreement with our previous postmortem analyses of calbindin- and parvalbumin-IR populations of GABAergic neurons in different regions of prefrontal cortex (8). Marked 50% reductions in the density of CB-IR neurons were detected in area 9 of the dlPFC but not in area 47 of the ventral orbitofrontal cortex (ORB) in MDD subjects. Thus, both postmortem and neuroimaging studies suggest that GABA pathology in

MDD is widespread, but regionally specific. The findings suggest that a decreased density of CB-IR GABA neurons in the occipital cortex (present study) and dlPFC (8) in MDD subjects may contribute to the low cortical GABA content observed in these cortical regions (2,3,4,5, 6) in depressed patients.

Although parvalbumin-IR was not specifically examined in this study, the subpopulation of parvalbumin-IR GABAergic neurons was not affected in either dlPFC or ORB in previous studies (8). The calbindin- and parvalbumin-IR neurons belong to two distinct subpopulations of GABA neurons. Calcium binding protein, calbindin, is mainly expressed by double bouquet neurons, which make synapses on dendrites and synaptic spines of pyramidal neurons and have physiological features of non fast spiking interneurons. In contrast, GABA neurons expressing parvalbumin correspond to basket or chandelier cells, form synaptic connections on somata and axonal initial segment of pyramidal neurons, as well as have physiological properties of fast-spiking interneurons (21). Thus suggesting a specific loss of a physiologically unique cell type may be associated with the pathology underlying MDD. Based on our postmortem findings CB-IR subpopulation of GABAergic neurons is affected in MDD, whereas other subpopulations have yet to be studied.

The present study, to our knowledge provides the first postmortem evidence for GABA neurons pathology in the occipital cortex in depression. Although visual function is not commonly studied in association with depression, intriguing subjective complaints of visual deficits (22) and neurofunctional alterations in the occipital lobe have been reported in patients with MDD. Specifically, differences in visual evoked potential response amplitudes (23,24) and stimulus-induced plasticity of VEP responses (25) were previously observed between healthy control subjects and in subjects with severe MDD. Moreover, contrast discrimination thresholds were found to be altered in patients with MDD compared to comparison subjects (26), and a recent study by Bhawagar *et al.* (27) demonstrated that spatial suppression for high contrast stimuli (postulated to be mediated by GABAergic interneurons) is abnormal in MDD subjects compared to healthy comparison subjects. These studies suggest pathophysiological processes within the occipital cortex may in fact be associated with MDD. Interestingly, the parieto-occipital cortex is also one of the brain regions recently identified in the processing of sustained anxiety (28).

In sum, this preliminary study demonstrates a reduction in the density of CB-IR neurons in the occipital cortex of individuals suffering with MDD. These findings are consistent with several reports of decreased GABA content in the occipital cortex and further suggest that the underlying pathology associated with MDD may be more widespread in the brain than commonly conceived. The present study however, has some limitations. The sample size used in our study is relatively small and further investigations with increased number of subjects could provide better understanding of the influence of potentially confounding variables (medication use, smoking status or alcohol abuse) on our results and demonstrate the consistency of the finding.

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Figure 1.

a: Nissl stained section of a non-psychiatric control subject showing the typical cytoarchitectonic features of area 17. **b:** CB immunoreactive neurons in control subject (adjacent section to Nissl). **c:** CB immunoreactive neurons in MDD subject. Note that the majority of CB-IR neurons are localized to layer II and in a smaller number to the upper part of layer III. Images were obtained using the $\times 4$ objective, scale bar = 125 μm .

Figure 2.

Cell packing density of CB-IR neurons in the occipital cortex (Brodmann's area 17) of control and MDD subjects. Values for the individual subjects (circles) and mean values (horizontal lines) are presented.

Figure 3.

Cell packing density of CB-IR neurons in the occipital cortex (Brodmann's area 17) of control, MDD suicide (MDDs) and MDD non suicide (MDD ns). Values for the individual subjects (circles) and mean values (horizontal lines) are presented. Note that the mean values for CB-IR neurons density are comparable between MDD suicide and MDD non suicides (unpaired t-test, $t=0.048$, $df=8$, $p=0.96$; Mann Whitney test, $Z=0.0$, $p=1$).

Figure 4. Size of CB-IR neurons in the occipital cortex (Brodmann's area 17) of control and MDD subjects. Values for the individual subjects (circles) and mean values (horizontal lines) are presented.

Table 1

Demographic characteristics of control and MDD subjects

Parameter	Controls (n=10)	MDD (n=10)
Age (years)	40.2 ± 4.9	44.8 ± 5.3
Age range (years)	17 – 65	19 – 81
PMI (hrs)	22.5 ± 2.1	24.2 ± 1.3
PMI range (hrs)	13 – 35	17 – 31
pH	6.6 ± 0.09	6.49 ± 0.08
pH range	5.93 – 7.0	6.06 – 6.82
TF (months)	44.2 ± 3.5	53.7 ± 4.8
TF range (months)	22 – 57	38 – 82
Gender (F:M)	4:6	4:6
Medication history *	none	n = 3
Toxicology:		
• Clean	n=8	n=3
• Antidepressant drugs	none	n=1 (sertraline)
• Other	n=2 (propoxyphene, oxycodone, n=1; ethanol, n=1)	n = 6 (morphine, codeine, hydrocodone, diphenhydramine, n=1; lidocaine, n=1; tramadol, n=1; cannabinoids, n=1; ethanol, CO, alprazolam, n=1; ethanol, n=1)
Cause of death	Cardiovascular disease, n=10	Suicide, n=6 (shot gun, n=3; CO poisoning, n=1; hanging, n=1; drowning, n=1), Other causes, n=4 (cardiovascular disease, n=3; homicide, n=1)
Diagnosis	none (n=10)	MDD, n=8; MDD and alcohol abuse, n=1; MDD and cannabis abuse, n=1
Duration of MDD (years)	not applicable	6 ± 2
Duration range	not applicable	1 month – 20 years

Parameter	Controls (n=10)	MDD (n=10)
Onset of MDD (years)	not applicable	36.7 ± 5.1
Onset range (years)	not applicable	15 – 70
Smoking	Smokers, n=1	Smokers, n=6

MDD - major depressive disorder; PMI - postmortem interval; TF - time in formalin; CO - carbon monoxide.

* Treatment with antidepressants within 4 weeks prior to death.

Data represent the mean ± SEM. The average age, PMI, TF and pH of MDD subjects were not statistically different from the control subjects.

Table 2
Summary of correlations (Pearson analyses) between the density and size of CB-IR neurons and confounding variables in control and MDD group

Variable	Density				Size			
	Control		MDD		Control		MDD	
	r	p	r	p	r	p	r	p
Age at the time of death	-0.073	0.841	-0.356	0.313	0.147	0.686	0.276	0.44
Age at onset of depression	-	-	0.481	0.16	-	-	0.213	0.556
PMI	0.539	0.108	-0.193	0.144	-0.178	0.624	0.091	0.803
pH	0.36	0.307	0.16	0.659	-0.326	0.358	0.427	0.219
TF	0.262	0.465	0.497	0.144	-0.002	0.996	0.026	0.942

MDD - major depressive disorder; PMI - postmortem interval; TF - time in formalin