Neuroscience 316 (2016) 209–220

DENSITY OF GFAP-IMMUNOREACTIVE ASTROCYTES IS DECREASED IN LEFT HIPPOCAMPI IN MAJOR DEPRESSIVE DISORDER

J. A. COBB,[a†] K. O'NEILL,[a†] J. MILNER,[a] G. J. MAHAJAN,[a] T. J. LAWRENCE,[a] W. L. MAY,[b] J. MIGUEL-HIDALGO,[a] G. RAJKOWSKA[a] AND C. A. STOCKMEIER[a,c*]

[a] *Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216, USA*

[b] *School of Health Related Professions, University of Mississippi Medical Center, Jackson, MS 39216, USA*

[c] *Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106, USA*

Abstract—Neuroimaging and postmortem studies of subjects with major depressive disorder (MDD) reveal smaller hippocampal volume with lengthening duration of illness. Pathology in astrocytes may contribute significantly to this reduced volume and to the involvement of the hippocampus in MDD. Postmortem hippocampal tissues were collected from 17 subjects with MDD and 17 psychiatrically-normal control subjects. Sections from the body of the hippocampus were immunostained for glial fibrillary acidic protein (GFAP), a marker of intermediate filament protein expressed in astrocytes. The density of GFAP-immunoreactive astrocytes was measured in the hippocampus using 3-dimensional cell counting. Hippocampal subfields were also assessed for GFAP-immunoreactive area fraction. In CA1, there was a significant positive correlation between age and either density or area fraction in MDD. The density of astrocytes in the hilus, but not CA1 or CA2/3, was significantly decreased only in depressed subjects not taking an antidepressant drug, but not for depressed subjects taking an antidepressant drug. The area fraction of GFAP-immunoreactivity was significantly decreased in the dentate gyrus in women but not men with depression. In CA2/3, the area fraction of GFAP-immunoreactivity was inversely correlated with the duration of depression in suicide victims. Astrocyte contributions to neuronal function in the hilus may be compromised in depressed subjects not taking antidepressant medication. Due to the cross-sectional nature of the present study of postmortem brain tissue, it remains to be determined whether antidepressant drug treatment prevented a decrease in GFAP-immunoreactive astrocyte density or restored cell density to normal levels.

© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: hippocampus, major depressive disorder, postmortem, astrocyte, GFAP.

INTRODUCTION

Major depressive disorder (MDD) is a serious, debilitating illness and a substantial contributor to global burden of disease (Whiteford et al., 2013). In 2010, MDD accounted for 8.2% of years lived with disability and 2.5% of disability-adjusted life years (DALY) globally with particular impact on adults of working age (Ferrari et al., 2013). Additionally, MDD increased the risk of both suicide and ischemic heart disease, accounting for 16 million and 4 million outcome-related DALY, respectively (Ferrari et al., 2013). Medications to alleviate depressive symptoms have been available for more than 50 years, but efficacy has improved a little, with no more than two thirds of patients achieving partial or full recovery (Little, 2009). Improved knowledge of the underlying pathophysiology of this disorder is critical to the development of more effective treatments.

Neuroimaging studies consistently reveal smaller hippocampal volume in MDD (Sheline et al., 1996; Bremner et al., 2000; Lorenzetti et al., 2009; Kempton et al., 2011; Brown et al., 2014; Schmaal et al., 2015). A meta-analysis of 32 magnetic resonance imaging (MRI) studies suggests about a 4% smaller left hippocampal volume in patients with a history of multiple episodes of depression or duration of illness exceeding 2 years (McKinnon et al., 2009). Our group recently published a stereological assessment of the volume of the postmortem hippocampus in chronic/recurrent depression and noted that the total volume was decreased with increasing duration of illness (Cobb et al., 2013). We sought to determine whether the change in volume in MDD was due to altered neuronal or glial number, density or soma size. Although there was no difference in the total number of neurons or glia, glial nuclear volume was increased with age in CA1 in depressed subjects and in the dentate gyrus of those who died by suicide, which suggests pathology in at least one glial cell type (Jorgensen et al., 2007; Webster et al., 2009; Walters et al., 2012). Nissl staining was used in Cobb et al. (2013) to identify glial cell nuclei but was not used to

*Correspondence to: C. A. Stockmeier, Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA. Tel: +1-601-984-6675; fax: +1-601-984-5899.

E-mail address: cstockmeier@umc.edu (C. A. Stockmeier).

† Contributed equally to the manuscript.

Abbreviations: ANCOVA, analyses of covariance; BDNF, brain-derived neurotrophic factor; CUS, chronic unpredictable stress, DALY, disability-adjusted life years; EAAT, excitatory amino acid transporter; FGF2, fibroblast growth factor 2; GFAP, glial fibrillary acidic protein; HPA, hypothalamic–pituitary–adrenocortical; MDD, major depressive disorder.

http://dx.doi.org/10.1016/j.neuroscience.2015.12.044

0306-4522/© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

differentiate glial cell type. Astrocytes, being the most numerous glial cell type in CNS and possessing relatively large cell bodies and extensive processes, may contribute significantly to the reduction in hippocampal volume with increasing duration of MDD.

Astrocytic pathology is implicated in the pathophysiology of MDD (Rajkowska and Stockmeier, 2013). Treatments effective in MDD promote astrocyte proliferation and gene expression (Fujiki and Steward, 1997; Jansson et al., 2009; Li et al., 2009; Liu et al., 2009), whereas a glial toxin-induced reduction of the astrocyte population in medial prefrontal cortex induces a depressive-like phenotype in rats (Banasr and Duman, 2008). Astrocyte density and glial fibrillary acidic protein (GFAP) immunoreactivity are altered in an age-dependent manner in postmortem dorsolateral prefrontal cortex, with cell density and immunoreactivity, as well as expression of GFAP protein, lower in younger MDD subjects as compared to age-matched control subjects (Miguel-Hidalgo et al., 2000; Si et al., 2004). Lower levels of GFAP were also observed in postmortem orbitofrontal cortex from younger adults with MDD, as compared to age-matched control subjects, but only in those depressed subjects without an antidepressant medication in blood at the time of death (Miguel-Hidalgo et al., 2010). The expression of both GFAP mRNA and protein was reduced in astrocytes isolated by laser-capture microdissection from postmortem locus coeruleus of subjects with MDD (Chandley et al., 2013). In prefrontal cortex, mRNA expression for a number of astrocyte-related genes, including GFAP, was significantly decreased in depressed suicide victims (Nagy et al., 2015). Moreover, in those depressed suicides with astrocytic dysfunction, methylation associated with astrocyte-related genes was also decreased (Nagy et al., 2015). In a follow-up study of subjects in Nagy et al. (2015), Torres-Platas et al. (2015) observed that expression of GFAP protein and mRNA was significantly decreased in mediodorsal thalamus and caudate nucleus, but not in primary motor or visual cortex or cerebellar cortex of depressed suicides. Taken together, these data suggest region-specific astrocytic pathology in MDD.

Astrocytic pathology in depression is less well studied in the human hippocampus, but the clinical and preclinical literature suggests involvement of hippocampal astrocytes in depression and its treatment (Müller et al., 2001; Gosselin et al., 2009; Araya-Callís et al., 2012; Gos et al., 2013; Zhang et al., 2015). Willard et al. (2013) noted a decrease in the total number of glial cells in the anterior but not posterior hippocampus in a non–human primate model related to depression. In chronic unpredictable stress (CUS), a rodent model for the induction of depression-related behaviors, the antidepressant drug clomipramine reversed the effects of CUS on protein and mRNA expression of GFAP in the hippocampus (Liu et al., 2009). In a recent review, Czéh and Di Benedetto (2013) note that antidepressant drugs affect a variety of astrocyte-related proteins and mRNA expression in the hippocampus, as well as gliogenesis.

Unbiased, design-based 3-dimensional cell counting was used here in formalin-fixed postmortem left hippocampus to compare GFAP-immunoreactive (-ir) astrocyte density between MDD and normal control subjects that were age- and sex-matched. In addition, GFAP-ir area fraction was measured as a semi-quantitative, two-dimensional index of the extent of astrocytic processes using the same tissue sections used for assessing astrocyte density. We examined GFAP-ir astrocytes in the rostral body of the left hippocampus because of our parallel studies on gene expression in this region on the right side showing reduced expression of astrocyte-related genes in MDD (Duric et al., 2010, 2013). In light of observations of reduced density of GFAP-ir astrocytes in the prefrontal cortex in MDD, it is hypothesized that astrocyte packing density and area fraction are decreased in the hippocampus in MDD.

EXPERIMENTAL PROCEDURES

Subjects

Tissues were collected at autopsy at the Cuyahoga County Medical Examiner's Office, Cleveland, OH, and the cause of death was ruled by the Medical Examiner. The protocol for recruitment, tissue collection, and interviews was approved by the institutional review boards of University Hospitals of Cleveland and the University of Mississippi Medical Center. Written informed consent was obtained from legally-defined next-of-kin for tissue collection and informant-based retrospective diagnostic interviews. Cases with history or evidence of neurological injury or disorder were excluded.

The Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1995) was administered by a trained interviewer to next-of-kin for all subjects to retrospectively assess the presence or absence of Axis I diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (APA, 1994). Interview notes and clinical histories were reviewed independently by two licensed mental health clinicians, who assigned consensus diagnoses in conference. There is a high degree of agreement between diagnoses of MDD-based on interviewing next-of-kin and diagnoses based on examination of living subjects (DeJong and Overholser, 2009).

For tissue processing and microscopic analysis, each subject diagnosed with MDD ($n = 17$) was yoked with a control subject matched for sex, age (± 5 years), postmortem interval ($\pm \sim 7.5$ h), and tissue pH. There was no significant difference between the MDD and control cohorts in age, postmortem interval, tissue pH, or fixation time in formalin (Table 1). Urine and blood collected at autopsy were examined by the medical examiner for the presence of psychotropic medications or psychoactive substances. Laboratory personnel were unaware of individual diagnoses throughout the study. Clinical characteristics of the MDD cohort are summarized in Table 2. These subjects are identical to those studied in the related publication (Cobb et al., 2013). For details on individual subjects, see Tables 3–5.

All subjects with MDD met criteria for depression within the last two weeks of life except one subject who was in remission. No control subject met criteria for any current or past mood disorder or other psychiatric

Table 1. Demographic and histological characteristics of the subjects

	Control $n = 17$	Major depressive disorder $n = 17$	
Age (years)	51.8 ± 3.4	51.5 ± 3.1	$p = 0.950$
Sex (M/F)	13/4	12/5	–
Postmortem interval (PMI; h)	23.4 ± 1.5	24.4 ± 2.4	$p = 0.811$
Tissue pH	6.6 ± 0.1	6.4 ± 0.1	$p = 0.119$
Fixation time in formalin (weeks)	155 ± 21	127 ± 15	$p = 0.290$
Storage time in ethanol (days)	157 ± 25	117 ± 21	$p = 0.258$

Values are mean ± S.E.M. Demographic and histological characteristics did not statistically differ between control subjects and those with major depressive disorder.

Table 2. Clinical characteristics of the subjects

	Major depressive disorder $n = 17$
Age of onset of depression (years)	42.3 ± 4.2
Duration of depression (years)	9.0 ± 2.3
Single episode/recurrent or Chronic	6/11
Suicide (Y/N)	10/7
Antidepressant-detected postmortem (Y/N)	7/10

Values are mean ± S.E.M.

disorder. However, one control subject was in full remission for 10 years from a diagnosis of alcohol dependence. Similarly, one MDD subject had a history of alcohol dependence, also in full remission. At the time of death, three subjects with MDD also met criteria for sedative and alcohol abuse, cannabis abuse, or cannabis dependence.

Tissue preparation and immunohistochemistry

The left temporal lobe was collected at autopsy and submerged in phosphate-buffered formalin (4%). Tissues remained in formalin for 21–369 weeks (141.1 ± 13.1 weeks, mean ± S.E.M.). Temporal lobes were cut into 6-mm-thick slabs in the coronal plane, embedded in celloidin, sectioned on a microtome at a thickness of 40 μm, and stored in 70% ethanol.

GFAP was labeled in tissue sections stored in ethanol using immunohistochemistry to identify astrocytes. Adjacent sections were stained for Nissl substance using cresyl violet as a guide for delineating hippocampal subfields in the GFAP-ir sections (see Fig. 1). Storage time in ethanol did not significantly differ between MDD and control subjects (Table 1). For each subject, three tissue sections within the rostral body of the hippocampus located 400 μm apart were selected from ethanol storage and processed for celloidin removal and GFAP immunohistochemistry as described (Miguel-Hidalgo and Rajkowska, 1999). Free-floating sections were washed in Tris-buffered saline (TBS; pH 7.6) and processed using a primary antibody to GFAP diluted 1:500 (mouse monoclonal anti-GFAP, Sigma–Aldrich, St. Louis, MO, USA). A secondary antibody (biotinylated horse anti-mouse, included in VECTASTAIN® Elite® ABC kit [universal], Vector Laboratories, Burlingame, CA, USA) diluted 1:200 was used, and immunoreactivity was revealed by the ABC method using 3′-3′-diaminoben zidine tetrahydrochloride enhanced with nickel ammonium sulfate. GFAP-immunolabeled sections were mounted on glass slides and cover-slipped. Immunostaining was not detected in tissue sections in which the primary antibody was omitted.

Table 3. Features of control subjects

Age (years)	Sex – Race	Cause of death	PMI (h)	Tissue pH	Toxicology
66	M – AA	CVD	12.5	6.69	*n.d.*
65	F – Cauc	CVD	26	6.18	*n.d.*
61	M – Cauc	Atherosclerotic CVD	16	6.72	*n.d.*
32	M – Cauc	Blunt trauma to head and body	25	6.88	*n.d.*
43	M – Hisp	Crush injuries to head and body	21.8	6.58	*n.d.*
53	M – Cauc	Gastric hemorrhage	26.5	6.58	Meperidine, promethazine, morphine
50	M - Cauc	Atherosclerotic CVD	22.5	6.78	*n.d.*
56	M – Cauc	Blunt trauma to head and body	27.2	6.86	Pseudoephedrine
60	F – Cauc	Atherosclerotic CVD	17.2	.	*n.d.*
54	M – Cauc	Post-surgical complication	26.5	6.50	Morphine
62	M – Cauc	MCI	21	.	*n.d.*
18	M – AA	Bronchial asthma	31.2	6.40	Midazolam
56	F – AA	Hypertrophic cardiomyopathy	16.8	6.09	*n.d.*
65	M – Cauc	CVD	22.2	6.60	*n.d.*
61	M – AsA	CVD	30.5	6.61	Lidocaine
50	F – AA	Hypertrophic cardiomyopathy	20	6.81	*n.d.*
28	M – AA	Bronchial asthma	35.5	6.32	*n.d.*

Sex-race: F, female; M, male; AA, African-American; AsA, Asian-American; Cauc, Caucasian; Hisp, Hispanic. Cause of death (Type): CVD, cardiovascular disease; MCI, myocardial infarction. Tissue pH: ".", frozen tissue not available. Toxicology: CO, carbon monoxide; *n.d.*, nothing detected.

Table 4. Features of subjects with major depressive disorder (MDD)

Age (years)	Sex – Race	Cause of death (Type)	PMI (h)	Tissue pH	Toxicology	Current psychotropic prescription
79	M – Cauc	electrocution (S)	21.5	.	Sertraline	Sertraline, temazepam
65	M – Cauc	SIGSW (S)	30	6.24	Codeine	Unknown
41	M – Cauc	CO poisoning (S)	16	.	Ethanol, CO	None
52	M – AA	SIGSW (S)	10	.	*n.d.*	None
48	M – Cauc	Aortic dissection	16.5	6.26	*n.d.*	None
48	F – Cauc	Hanging (S)	6.5	.	Citalopram, trazodone	Quetiapine, oxcarbazepine, aripiprazole, escitalopram
49	M – Cauc	Atherosclerotic coronary artery disease with cardiomegaly	27.5	.	Diazepam, desipramine, lidocaine	Imipramine, diazepam
19	M – Cauc	SIGSW (S)	24.2	6.82	Cannabinoids	Fluoxetine, amphetamine/ dextro-amphetamine
61	F – Cauc	SIGSW (S)	37.8	6.66	*n.d.*	None
56	F – Cauc	Acute bronchopneumonia, DM	38	5.61	Doxylamine	None
54	M – Cauc	Atherosclerotic coronary artery disease, squamous cell carcinoma	38	6.54	Fluoxetine, dextromethorphan	Fluoxetine
50	F – Cauc	Atherosclerotic CVD	28.5	6.47	Dextromethorphan	Imipramine
51	M – Cauc	Hypertensive and atherosclerotic cardiomyopathy	11.5	6.42	Lorazepam, bupropion, citalopram	Bupropion, citalopram, lorazepam, temazepam
44	F – Cauc	SIGSW (S)	29	6.71	Bupropion, diphenhydramine, venlafaxine, hydrocodone	Bupropion, venlafaxine, hydrocodone
59	M – Cauc	CO poisoning (S)	26.8	6.22	CO, fluoxetine	Fluoxetine
37	M – AA	SIGSW (S)	31.5	6.71	Ethanol	None
62	M - AA	CVD, obesity, emphysema	22	6.06	*n.d.*	None

Sex-race: F, female; M, male; AA, African-American; Cauc, Caucasian. Cause of death (Type): S, suicide; CO, carbon monoxide; CVD, cardiovascular disease; DM, diabetes mellitus; SIGSW, self-inflicted gunshot wound. Tissue pH: ".", frozen tissue not available. Toxicology: CO, carbon monoxide; *n.d.*, nothing detected.

Table 5. Clinical characteristics of subjects with Major Depressive Disorder (MDD)

Age of onset of MDD (years)	Duration of MDD (years)	Single-episode, recurrent, or chronic MDD
79	0.17	Single
65	0.08	Single
41	0.33	Single
55	0.25	Single
39	6	Recurrent
20	20	Recurrent
47	10	Chronic
14.5	4.5	Recurrent
49	12	Recurrent
55	1	Single
32.5	20	Recurrent
32.5	15	Chronic, in remission last few years
16	35	Chronic
33	11	Recurrent
54	5	Recurrent
37	0.08	Single
49	13	Recurrent

Estimation of astrocyte density and area fraction

In each tissue section, contours outlining sub-regions of the hippocampal formation were outlined as described in Cobb et al. (2013) and were traced with a Nikon Eclipse 80i microscope (Nikon Instruments, Melville, NY, USA) using Stereo Investigator® software (version 7.0, MBF Bioscience, Williston, VT, USA). Three-dimensional estimates of cell density of GFAP-ir astrocytes were made in CA1, CA2/3, and hilus using the Optical Fractionator method in Stereo Investigator. Hilus refers to the polymorphic cell layer plus the portion of CA3 located within the concavity of the dentate gyrus (Fig. 1A). Astrocyte density was not assessed in dentate gyrus due to high packing density preventing the discernment of individual astrocyte cell bodies, even under high magnification. Cell counting was performed elsewhere in the hippocampus at 400× magnification using a Nikon Plan Apo 40 × oil-immersion objective (N.A. = 1.0, W.D. = 0.16 mm). Sampling parameters are summarized in Table 6.

Area fraction of GFAP immunoreactivity was measured in CA1, CA2/3, and DG. For methods, see Miguel-Hidalgo et al. (2000, 2010). Area fraction was not measured in hilus due to saturation of GFAP-ir signal

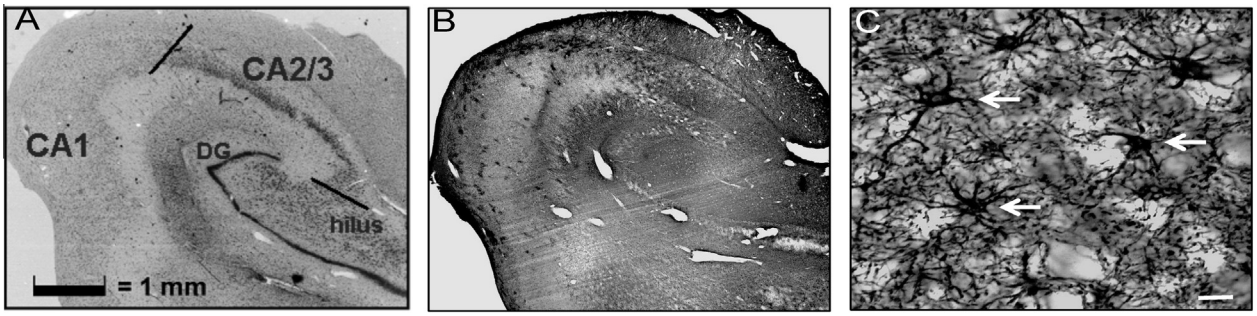

Fig. 1. Photomicrographs of subregions of the human hippocampus. Nissl-stained section of the hippocampus (A) with adjacent GFAP-labeled section (B). High-magnification image of GFAP-immunoreactive astrocytes in human hippocampus (C). Representative astrocytes noted by white arrows. Scale bar in C = 10 μm. *Abbreviation:* DG = dentate gyrus.

Table 6. Parameters for determining astrocyte density

	Disector dimensions (μm)	Grid dimensions (μm)
CA1	225 (X) × 140 (Y) × 25 (Z)	250 (X) × 250 (Y)
CA2/3	215 (X) × 130 (Y) × 9 (Z)	250 (X) × 250 (Y)
Hilus	150 (X) × 150 (Y) × 9 (Z)	300 (X) × 300 (Y)

there, even though astrocyte cell bodies could be easily identified. Area fraction was measured by defining a non-immunoreactive background in the stratum radiatum and calculating the percent of area of the hippocampal gray matter with immunoreactive gray level greater than the background level.

For each hippocampal field in which area fraction was examined, a series of non-overlapping images covering the entire surface area of the hippocampus was captured using the 'acquire image' function in Stereo Investigator and saved in tagged image file format (TIFF). Each image was opened in Image J software (National Institutes of Health, Bethesda, MD, USA), converted to 8-bit format, and any portion of the hippocampal field of interest within the image was outlined. GFAP-ir area and total area within the outlines were measured using the threshold feature in Image J with the defined non-immunoreactive background serving as reference. For each hippocampal field, the sum of immunoreactive areas was divided by that for the total area and this quotient was multiplied by 100 to yield the percent GFAP-ir area fraction. All values presented below are mean ± S.E.M.

Statistical analyses

Sample size was determined by power analyses (Lenth RV, 2006–2009) based on previously reported differences in astrocyte density and GFAP-immunoreactivity in depression (Miguel-Hidalgo et al., 2000, 2010). All subsequent statistical analyses were performed using the SAS v. 9.2 software package (SAS Institute, Cary, NC, USA). The threshold for statistical significance was set at a Type I error rate of $\alpha = 0.05$; non-significant trends were noted at $0.05 \leqslant p \leqslant 0.10$.

Prior to hypothesis testing, dependent variables (*e.g.* CA1 astrocyte density) were tested by cohort for normality of distribution via the Shapiro-Wilk *W* statistic. Those variables for which the *W* statistic suggested a non-normal distribution in both cohorts underwent comparison by cohort via the (nonparametric) Wilcoxon rank sum test. Otherwise, cohort comparisons were made via (parametric) *t*-tests. Pooled *t*-tests were used if the folded *F* statistic indicated equality of variances between the two cohorts; Satterthwaite *t*-tests were used if folded *F* statistic suggested unequal variances.

Some discrete demographic variables pertain only to MDD subjects, including death by suicide, presence of an antidepressant medication in postmortem fluids, or recurrence of depressive illness. To test potential effects of these factors, the MDD cohort was divided accordingly into two groups, yielding a total of three groups to compare *vis-à-vis* each of those factors. Dependent variables were tested by these new groupings for normality of distribution, as done earlier by cohort, and comparisons were made between these two groups and controls using either analyses of variance (ANOVA) using the General Linear Model procedure or (nonparametric) Kruskal–Wallis tests. *Post hoc* comparisons for all ANOVA were made using Tukey's Honestly Significant Difference test, which preserves experiment-wise Type I error rate across multiple comparisons (Ott and Longnecker, 2001). *Post hoc* comparisons following (nonparametric) Kruskal–Wallis tests were made via Wilcoxon rank sum tests of all possible comparisons, with the Bonferroni correction applied to preserve experiment-wise Type I error rate across multiple comparisons.

Potential effects of sex or interactions thereof with cohort were assessed using a two-way factorial ANOVA (cohort × sex) or the equivalent analyses of covariance (ANCOVA) adjusting for age (see below). If Shapiro–Wilk tests indicated nonparametric statistics should be used, corresponding Kruskal–Wallis tests were used. Sex effects were not assessed for comparisons where the MDD cohort is subdivided into groups based on clinical variables (e.g. death by suicide or presence of antidepressant in toxicology) because this would result in there being too few subjects in those parsed groups to provide appropriate statistical power for meaningful analysis and conclusions.

For all ANCOVA conducted here, age was chosen for inclusion in the model through the examination of Pearson's correlations, across cohort, between

dependent variables and continuous demographic variables prior to conducting the ANCOVA. Other such variables assessed but ultimately not included in the ANCOVA included time between death and fixation of tissue (postmortem interval, PMI), tissue pH, fixation time in formalin, and storage time in ethanol. For all ANCOVA, *post hoc* comparisons were conducted using least-squares means. Pearson's correlations were examined by class variable for linear associations between dependent and continuous demographic variables.

RESULTS

GFAP-immunoreactive astrocyte density

Astrocyte density in CA1 alone increased with age in MDD ($r = 0.638$, $p = 0.006$) (Fig. 2A) but not in control subjects ($r = 0.362$, $p = 0.200$) (Fig. 2B). After adjusting for age, astrocyte density in the hilus was 26% lower in subjects with MDD in whom no evidence of antidepressant use was detected postmortem (MDD, no antidepressant, 3947 ± 329 cells/mm^3) than in control subjects (5304 ± 346 cells/mm^3) or subjects with MDD for whom postmortem fluids contained an antidepressant medication (MDD, with antidepressant, 5172 ± 526 cells/mm^3) (ANCOVA, df = 2, $F = 6.58$, $p = 0.005$) (Fig. 3). *Post hoc* pairwise comparisons revealed that astrocyte density was significantly decreased in MDD, no antidepressant, vs. control subjects ($p = 0.003$) and vs. MDD, with antidepressant, ($p = 0.011$).

There was no significant difference in astrocyte density between subjects with MDD and control subjects in CA1 or CA2/3, regardless of the presence of an antidepressant medication (data not included). Likewise, there was no difference in astrocyte density in any of the hippocampal regions between MDD subjects dying by suicide and those not dying by suicide (data not included).

GFAP-immunoreactive area fraction

GFAP-ir area fraction in CA1 increased with age in MDD ($r = 0.525$, $p = 0.030$) (Fig. 4A) but not in control subjects ($r = -0.361$, $p = 0.130$) (Fig. 4B).

After adjusting for age, there was a main effect of cohort by sex for area fraction in the dentate gyrus (ANCOVA, df = 3, $F = 3.10$, $p = 0.043$) (Fig. 5A). *Post hoc* pairwise comparisons revealed that GFAP area fraction was significantly decreased in females with MDD vs. males with MDD ($p = 0.009$), vs. male control subjects ($p = 0.009$), and a trend vs. female control subjects ($p = 0.070$).

In CA2/3, there was a trend for a similar main effect of cohort by sex (ANCOVA, df = 3, $F = 2.71$, $p = 0.064$), with area fraction again being lower in females with MDD (Fig. 5B).

There was no significant difference in GFAP-ir area fraction between MDD and control subjects in CA1, nor was there a significant difference in area fraction in any of the hippocampal regions between MDD subjects dying by suicide and those not dying by suicide (data not included). Nevertheless, in subjects with MDD that died by suicide, GFAP-ir area fraction significantly decreased with duration of depression in CA2/3 ($r = -0.687$, $p = 0.028$) (Fig. 6).

DISCUSSION

The packing densities of GFAP-ir astrocytes and immunoreactive area fraction were examined in tissue sections from postmortem hippocampus. Decreased astrocyte density was observed in hilus in subjects with MDD without (but not with) antidepressant treatment at the time of death. Astrocyte densities did not differ between MDD and control subjects in CA1 and CA2/3. However, astrocyte density and area fraction in CA1 increased with age only in MDD but not control subjects. Area fraction of GFAP-immunoreactivity was selectively decreased in dentate gyrus in females with MDD, with a trend toward that effect in females in CA2/3. In MDD subjects dying by suicide, area fraction in CA2/3 decreased with duration of depressive illness.

An interpretation of the main observation of decreased density in GFAP-ir astrocytes in hilus may involve mechanisms related to pathology in the dentate gyrus in MDD. We have identified reductions in expression of synapse- and glutamate-related genes in the dentate gyrus in MDD (Duric et al., 2010, 2013). Inasmuch as unmyelinated mossy fiber axons from granule cells of the dentate gyrus form multiple synapses onto pyramidal neurons restricted to the hilus and CA3 (Amaral and Lavenex, 2007), functional changes in mossy fiber axons traversing the hilus and CA3 in MDD may be accompanied by decreased density of GFAP-ir astrocytes. With astrocytes participating in the tripartite synapse, pathology in GFAP-ir astrocytes may interfere with uptake of potentially excitotoxic glutamate.

To date there are few published studies using postmortem tissues that examined astrocyte pathology in the hippocampus in MDD. One of the first, Müller et al. (2001) reported lower GFAP-ir astrocyte density in CA1 and CA2 but not CA3, CA4, or dentate gyrus in a semi-quantitative, two-dimensional study of postmortem human hippocampus from subjects with a mood disorder. No data were provided by Müller et al. (2001) as to whether antidepressant medications were detected in these subjects in postmortem blood. While the present study is not the first study published on GFAP-ir astrocytes in postmortem hippocampus in MDD, it is among the first using a three-dimensional cell counting method to do so. A recent study of postmortem brain tissue reported a lower density of astrocytes immunolabeled for S100B but not for GFAP in the pyramidal cell layer of CA1 in left and right posterior hippocampus of subjects with MDD (Gos et al., 2013). There are several differences between Gos et al. (2013) and the present study, such as the (1) level of the hippocampus examined, (2) length of postmortem interval, (3) clinical features of the subjects (e.g. in Gos et al., duration of depression was shorter and most MDD subjects were treated with an antidepressant medication) and (4) an effect of age on GFAP-ir astrocyte density in CA1 in the present study. It

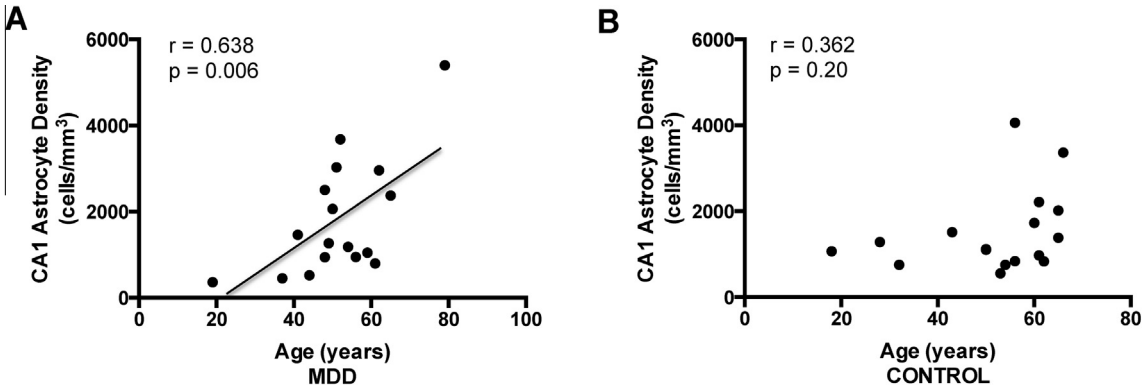

Fig. 2. Correlation between age and GFAP-immunoreactive astrocyte density in the CA1 region of the hippocampus. There was a positive, significant correlation between astrocyte density and age in subjects with major depressive disorder (MDD) (A). There was no significant correlation between astrocyte density and age in control subjects (B).

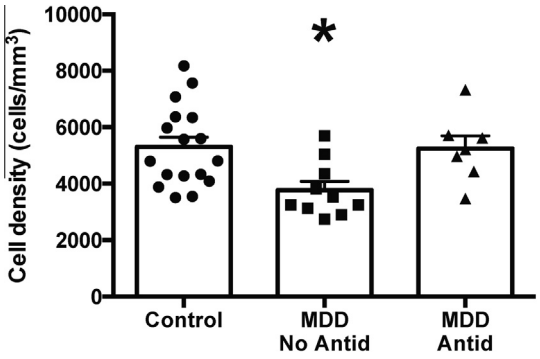

Fig. 3. GFAP-immunoreactive astrocyte density in the hilus. Astrocyte density was significantly decreased in subjects with major depressive disorder (MDD) with no antidepressant drug present postmortem (MDD, No Antid) compared to either control subjects or subjects with MDD with an antidepressant drug present postmortem (MDD, Antid). *ANCOVA, df = 2, $F = 6.58$, $p = 0.005$. $p = 0.003$ vs. control subjects and $p = 0.011$ vs. MDD, Antid. Histograms represent mean ± S.E.M.

remains to be determined if the S100B antibody labels a population of astrocytes not labeled by the GFAP antibody or if it labels a subpopulation of astrocytes that are also labeled by the GFAP antibody. Recently, using mostly the same depressed subjects as in Gos et al. (2013), Malchow et al. (2015), using Nissl plus myelin staining, examined the cell number and density of the total population of astrocytes in the left and right posterior hippocampus. There were no significant changes in either astrocyte number or density in CA4 ("hilus" in the present study) in MDD as compared to control subjects. Lack of a significant change in astrocyte number or density in the presence of antidepressant medications at the time of death in both Gos et al. (2013) and Malchow et al. (2015) is consistent with the present study where there was no significant difference in the density of GFAP-ir astrocytes between antidepressant-treated subjects with MDD and control subjects.

In Wistar–Kyoto rats, a model for anxiety and depression showing altered behavioral and endocrine responses to stressors, double immunofluorescence revealed that many S100B-immunopositive cells in the hippocampus lacked GFAP-immunoreactivity, unlike in Sprague–Dawley rats, where S100B and GFAP immunolabeling are co-localized in most astrocytes (Gosselin et al., 2009). However, it cannot be excluded that the reduction in density of S100B immunolabeled astrocytes in CA1 in MDD may have been due to the antidepressant treatment received in the 90 days prior to

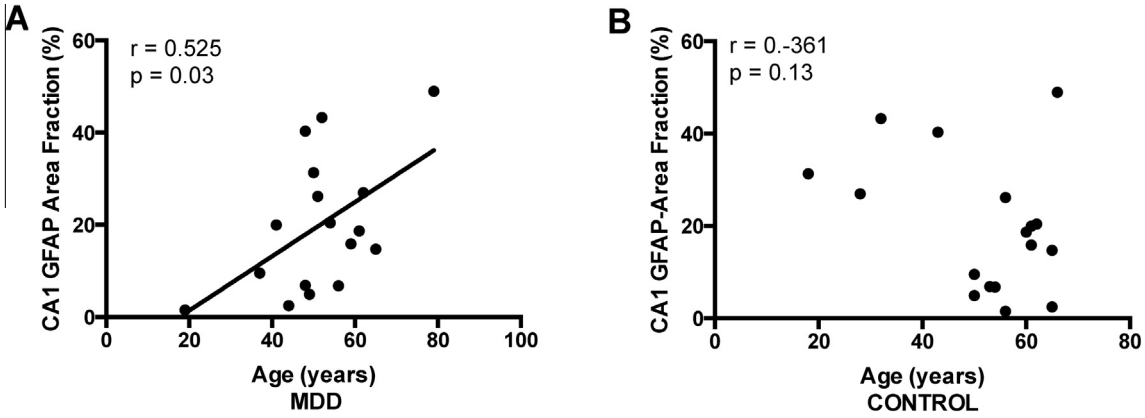

Fig. 4. Correlation between age and GFAP-immunoreactive area fraction in the CA1 regions of the hippocampus. There was a positive, significant correlation between area fraction and age in subjects with major depressive disorder (MDD) (A) but not in control subjects (B).

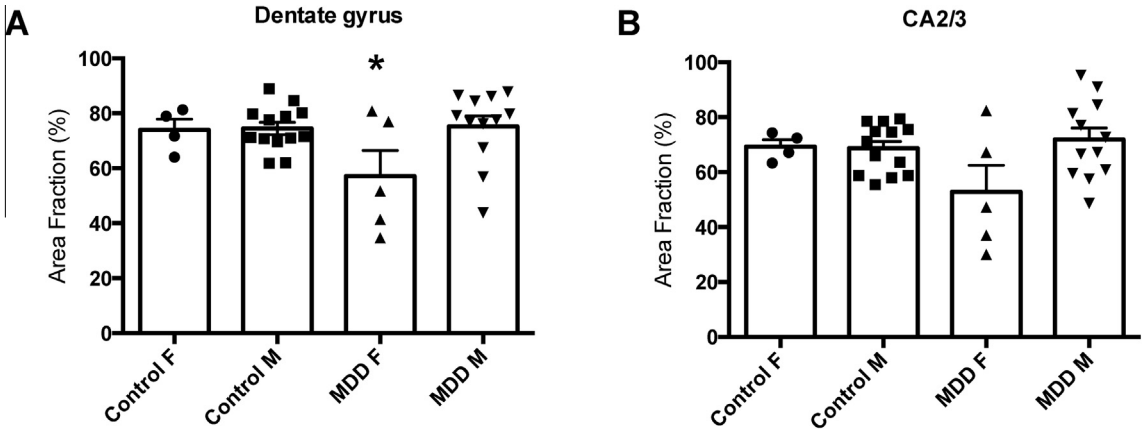

Fig. 5. GFAP-immunoreactive area fraction in control and major depressive disorder (MDD) men (M) and women (F). (A) After adjusting for age, there was a significant main effect of depression and gender in the dentate gyrus (ANCOVA, df = 3, $F = 3.10$, $p = 0.043$). *Females with MDD vs. males with MDD ($p = 0.009$), vs. male controls ($p = 0.009$), and a trend vs. female controls ($p = 0.07$). (B) After adjusting for age, there was a trend for a main effect of depression and gender in CA2/3 (ANCOVA, df = 3, $F = 2.71$, $p = 0.064$). Histograms represent mean ± S.E.M.

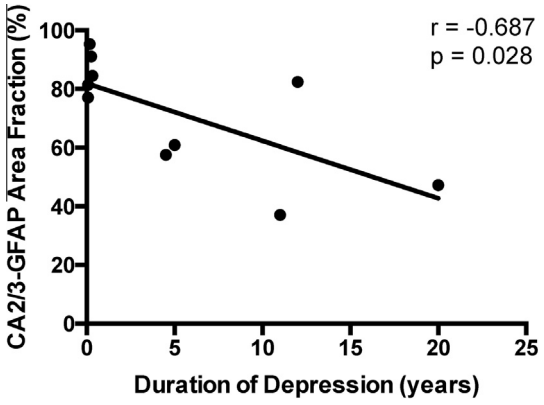

Fig. 6. Correlation between duration of MDD in suicide victims and GFAP-immunoreactive area fraction in CA2/3 region of the hippocampus. Area fraction was significantly decreased as a function of duration of illness.

death (Gos et al., 2013). In the hilus in the present study, the reduction in density of GFAP-ir astrocytes was only evident in subjects with MDD not taking an antidepressant medication at the time of death.

Age appears to have a significant effect on astrocyte density and area fraction of GFAP-immunoreactivity in depression. The observed increase reported here in MDD in astrocyte density and area fraction with age in CA1 is consistent with that noted by Miguel-Hidalgo et al. (2000) in dorsolateral prefrontal cortex. The increase in astrocytic markers in MDD with age may be in response to neuronal pathology reported in MDD in prefrontal cortex (Rajkowska et al., 1999, 2005) and hippocampus (Cobb et al., 2013).

Antidepressant medication appears to have an impact on GFAP-ir astrocytes in the hippocampus of depressed subjects. In the present study, subjects with MDD taking an antidepressant drug at the time of death demonstrated no significant change in astrocyte density in the hilus, whereas those not being treated with medication at the time of death showed a significant 26% decrease in cell density. In a tree shrew model of chronic psychosocial conflict, 35 days of this stress resulted in a reduction by 25% in the total number of hippocampal astrocytes (Czéh et al., 2006). Fluoxetine treatment initiated a week into the stress prevented the chronic stress-induced reduction in total astrocyte number. However, in a chronic psychosocial stress model in rats, citalopram, during the last four weeks of a five-week exposure to chronic stress, did not prevent a chronic stress-induced reduction of GFAP protein expression (Araya-Callís et al., 2012). Due to the cross-sectional nature of the present study of postmortem brain tissue, it cannot be determined whether antidepressant drug treatment prevented a decrease in astrocyte density or restored cell density to normal levels.

The relationship between suicide and/or MDD in the regulation of GFAP expression in astrocytes has not been clearly determined since most or all of the depressed subjects in these studies committed suicide (Miguel-Hidalgo et al., 2000, 2010; Kékesi et al., 2012; Gos et al., 2013; present study; exception is Müller et al. (2001) with only four of 15 depressed subjects dying by suicide). Consistent with the observations of Miguel-Hidalgo et al. (2000, 2010), Kékesi et al. (2012) also observed a decreased expression of the GFAP protein in prefrontal cortex in depressed suicide victims. In the present study, although only in subjects with MDD that died by suicide, area fraction of GFAP-immunoreactivity was significantly decreased in CA2/3 with increasing duration of depression. Additional studies are needed to confirm a relationship between duration of depression and expression of GFAP protein throughout the brain and whether the observation here is unique to suicide per se or suicide with depression.

In a small number of women (but not men) with MDD, area fraction of GFAP-immunoreactivity was significantly lower in the dentate gyrus, and there was a trend toward that effect in CA2/3. These observations might partially explain the greater incidence of MDD in women than men (Kessler et al., 1993), but these experiments need to be repeated in much larger cohorts of depressed and control women and men. Biological factors contributing

toward greater incidence of MDD in women than men likely involve effects of ovarian steroid hormones on brain development and function. The greater prevalence of MDD in women emerges in adolescence and persists until midlife, or around menopause (Cyranowski et al., 2000; Jans et al., 2007). Estrogen exerts antidepressant effects and both estrogen and progesterone influence psychological and endocrine responses to stressors, a risk factor for depression (Seeman, 1997; Young and Altemus, 2004; Österlund, 2010; Naninck et al., 2011). Estrogen affects gene expression via two cytosolic steroid receptors, estrogen receptors-α and -β (Katzenellenbogen et al., 2000), and both receptors are expressed in the hippocampus in neurons and in astrocytes (Österlund and Hurd, 2001; Lu et al., 2003; González et al., 2007). Transcription of GFAP mRNA is decreased in astrocytes co-cultured with neurons in response to estrogen and an estrogen response element is located in the 5'-upstream region of the GFAP promoter (Stone et al., 1998). Thus, fluctuations in estrogen levels may reduce astrocytic GFAP in women predisposed to or experiencing depressive illness.

Astrocytes in the hippocampus may be crucial for glucocorticoid-mediated feedback to the hypothalamic–pituitary–adrenocortical (HPA) axis. The glucocorticoid receptor-α protein is co-localized in approximately 20% of GFAP-ir astrocytes in dentate gyrus and 50% of those in CA subfields (Wang et al., 2013). Evidence for disruption of the HPA axis in MDD comes from studies reporting elevated morning cortisol and cortisol awakening response (Goldstein and Klein, 2014). Based on animal studies, elevated glucocorticoids may account for hippocampal anatomical and physiological pathology in depression and the deficits in GFAP-ir astrocytes reported here (Rodrigues et al. 2009; Carter et al., 2013; Zhang et al., 2015). In a semi-quantitative study of subjects with MDD or non-depressed subjects treated with steroids, astrocyte densities were lower in CA1 and CA2, but not CA3 or CA4 (hilus) in both clinical cohorts (Müller et al., 2001). The present study confirmed a decrease in GFAP-ir astrocytes in MDD, albeit in a different hippocampal subregion.

Excess glucocorticoids lead to increased glutamate neurotransmission, evidence of which in turn is observed in patients with MDD (Kugaya and Sanacora, 2005; Machado-Vieira et al., 2009; Zarate et al., 2010; Popoli et al., 2011). Astrocytes contribute significantly to glutamate reuptake. Impaired ability of astrocytes to take up excess extracellular glutamate via the excitatory amino acid transporters (EAAT) 1 and 2 is one possible mechanism by which astrocytic deficits may contribute to the pathophysiology of MDD. EAAT1 and EAAT2 are located on astrocyte cellular membranes of cell bodies and processes (Takahashi et al., 2015). Levels of EAAT1 and EAAT2 were decreased in the orbitofrontal cortex in MDD and in the hippocampus in animal models related to depression (Miguel-Hidalgo et al., 2010; Zink et al., 2010; Sanacora et al., 2012; Chen et al., 2014). Hence, even with normal numbers or densities of astrocytes, reductions in the extent of processes, as reflected by lower GFAP-ir area fraction, could thus contribute toward a vulnerability to stress and depression.

Trophic factors are a likely candidate linking glucocorticoid exposure to astrocyte density in MDD. Fibroblast growth factor 2 (FGF2) expression is reduced in postmortem hippocampus in MDD (Gaughran et al., 2006) but increases in hippocampal astrocytes with antidepressant treatment (Bachis et al., 2008) or voluntary physical exercise (Gómez-Pinilla et al., 1997). Peripheral administration of FGF2 in doses alleviating anxiety-like behavior is associated with increased survival of new neurons and astrocytes in rat hippocampus (Perez et al., 2009). However, glucocorticoids do promote FGF2 expression in cultured astrocytes (Gubba et al., 2004) and in hippocampal tissue of stressed rats (Frank et al., 2007).

The expression of brain-derived neurotrophic factor (BDNF), capable of regulating expression of GFAP, is altered in the hippocampus in depression and rodent models of chronic stress. BDNF protein and mRNA expression are down-regulated in postmortem hippocampus in MDD and suicide (Dwivedi et al., 2003; Duric et al., 2010). In addition, exposure to chronic stress or glucocorticoids decreases the expression of BDNF protein and mRNA in the rodent hippocampus (Duman and Aghajanian, 2012; Nowacka and Obuchowicz, 2013). Antidepressant-like effects of BDNF itself are well-documented, and they may involve direct effects of BDNF on astrocytes (Nowacka and Obuchowicz, 2013). BDNF infusion to the hippocampus restores astrocytic GFAP immunoreactivity and sucrose consumption that is reduced by CUS in rats (Ye et al., 2011). In turn, increased BDNF expression by hippocampal astrocytes is accompanied by alleviation of depressive-like behaviors in rodents and by increased neurogenesis (Quesseveur et al., 2013), neurogenesis being critical to the efficacy of antidepressant drugs (Santarelli et al., 2003; Perera et al., 2011; Samuels and Hen, 2011; Mateus-Pinheiro et al., 2013). Thus, the present report of decreased GFAP density in the hilus of antidepressant-free subjects with MDD may be in response to decreased levels of BDNF.

There are several limitations to the current study. Unlike studies of animal models or neuroimaging, research on postmortem tissues is not amenable to elucidating specific mechanisms underlying disease-related pathophysiology. Although studies of postmortem tissue have the advantage of allowing examination of subjects with depression as opposed to animal models designed to mimic outward signs thereof, another significant limitation is that subjects are examined only at a single point in time. While neuroimaging studies of live patients do lend themselves to longitudinal studies, unlike studies of postmortem tissues, however studies of actual tissue permit pathologies to be examined at the cellular level. This study of astrocyte density in mid-body of the hippocampus does not necessarily address whether there are differences in density in more rostral or caudal planes or in the total number throughout the entire hippocampus. Additionally, that not all astrocytes contain detectable GFAP is another limitation (Khakh and Sofroniew, 2015). Another limitation of the current study is not controlling for the variability in behavioral,

environmental, educational, and other socioeconomic factors inherent in human populations. Finally, care must be taken in over-interpreting our results regarding MDD after parsing our MDD cohort by treatment with antidepressants, death by suicide, duration of depression, or comparisons of age or sex, due to small sample size.

AUTHOR CONTRIBUTIONS

Craig A. Stockmeier designed the study and wrote the protocol, with critical assistance from Grazyna Rajkowska and Jose Miguel-Hidalgo, and supervised its execution. Justin A. Cobb assisted in designing the studies, conducted the area fraction analyses, and performed the statistical analyses with Warren May. Immunohistochemical assays were performed by Katie O'Neil and Thomas J. Lawrence. Katie O'Neil and Jessica Milner performed the density measures. Gouri J. Mahajan conducted celloidin embedding, sectioned all tissue specimens, performed Nissl staining, and supervised the immunohistochemical assays. All authors contributed to the drafting of this manuscript and have approved its final form.

DISCLOSURE

None of the authors have any actual, potential, or perceived financial, professional, or personal conflict of interest to declare.

Acknowledgments—The authors deeply appreciate the invaluable contributions made by the families consenting to donate brain tissue and be interviewed. We also gratefully acknowledge the support of the staff of the Cuyahoga County Medical Examiner's Office, Cleveland, Ohio. We acknowledge the expert assistance of Drs. James C. Overholser, George Jurjus and Lisa C. Konick, and of Lesa Dieter in establishing the psychiatric diagnoses, acquiring written consent and in collecting the tissues. For some of the subjects, the services of Timothy M. De Jong in acquiring written consent and Lisa Larkin and Nicole Herbst in tissue collection are gratefully acknowledged. This work was funded by support from The National Institute of Mental Health (MH67996) and the Imaging and Postmortem Brain Cores of the Center for Psychiatric Neuroscience, funded through an IDeA COBRE award from The National Institute of General Medical Sciences (P30 GM103328). These funding sources had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

REFERENCES

Amaral D, Lavenex P (2007) Hippocampal neuroanatomy. In: Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J, editors. The hippocampus book. New York: Oxford University Press. p. 37–114.

American Psychiatric Association (APA) (1994) Diagnostic and statistical manual of mental disorders. 4th ed. Washington, : APA.

Araya-Callís C, Hiemke C, Abumaria N, Flugge G (2012) Chronic psychosocial stress and citalopram modulate the expression of the glial proteins GFAP and NDRG2 in the hippocampus. Psychopharmacology 224:209–222.

Bachis A, Mallei A, Cruz MI, Wellstein A, Mocchetti I (2008) Chronic antidepressant treatments increase basic fibroblast growth factor and fibroblast growth factor-binding protein in neurons. Neuropharmacology 55:1114–1120.

Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry 64:863–870.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. Am J Psychiatry 157:115–118.

Brown ES, Hughes CW, McColl R, Peshock R, King KS, Rush AJ (2014) Association of depressive symptoms with hippocampal volume in 1936 adults. Neuropsychopharmacology 3:770–779.

Carter BS, Hamilton DE, Thompson RC (2013) Acute and chronic glucocorticoid treatments regulate astrocyte-enriched mRNAs in multiple brain regions in vivo. Front Neurosci 7:1–14.

Chandley MJ, Szebeni K, Szebeni A, Crawford J, Stockmeier CA, Turecki G, Miguel-Hidalgo JJ, Ordway GA (2013) Gene expression deficits in pontine locus coeruleus astrocytes in men with major depressive disorder. J Psychiatry Neurosci 38:276–284.

Chen JX, Yao LH, Xu BB, Qian K, Wang HL, Liu ZC, Wang XP, Wang GH (2014) Glutamate transporter 1-mediated antidepressant-like effect in a rat model of chronic unpredictable stress. J Huazhong Univ Sci Technolog Med Sci 34:838–844.

Cobb JA, Simpson J, Mahajan G, Overholser JC, Jurjus GJ, Dieter L, Herbst N, May W, Rajkowska G, Stockmeier CA (2013) Hippocampal volume and total cell numbers in major depressive disorder. J Psychiatr Res 47:299–306.

Cyranowski JM, Frank E, Young E, Shear MK (2000) Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. Arch Gen Psychiatry 57:21–27.

Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31:1616–1626.

Czéh B, Di Benedetto B (2013) Antidepressants act directly on astrocytes: evidences and functional consequences. Eur Neuropsychopharmacol 23:171–185.

DeJong TM, Overholser JC (2009) Assessment of depression and suicidal actions: agreement between suicide attempters and informant reports. Suicide Life Threat Behav 39:38–46.

Duman RS, Aghajanian GK (2012) Synaptic dysfunction in depression: potential therapeutic targets. Science 338:68–72.

Duric V, Banasr M, Licznerski P, Schmidt HD, Stockmeier CA, Simen AA, Newton SS, Duman RS (2010) A negative regulator of MAP kinase causes depressive behavior. Nat Med 16:1328–1332.

Duric V, Banasr M, Stockmeier CA, Simen AA, Newton SS, Overholser JC, Jurjus GJ, Dieter L, Duman RS (2013) Altered expression of synapse and glutamate related genes in postmortem hippocampus of depressed subjects. Int J Neuropsychopharmacol 6:69–82.

Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN (2003) Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Arch Gen Psychiatry 60:804–815.

Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJL, Vos T, Whiteford HA (2013) Burden of depressive disorders by country, sex, age, and year: Findings from the Global Burden of Disease Study. PLoS Med 10:e1001547.

First M, Spitzer R, Gibbon M, Williams J (1995) Structured clinical interview for the DSM-IV Axis I disorders (SCID patient edition), version 2.0. New York, NY: New York State Psychiatric Institute.

Frank MG, Der-Avakian A, Bland ST, Watkins LR, Maier SF (2007) Stress-induced glucocorticoids suppress the antisense molecular regulation of FGF-2 expression. Psychoneuroendocrinology 32:376–384.

Fujiki M, Steward O (1997) High frequency transcranial magnetic stimulation mimics the effects of ECS in upregulating astroglial gene expression in the murine CNS. Mol Brain Res 44:301–308.

Gaughran F, Payne J, Sedgwick PM, Cotter D, Berry M (2006) Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. Brain Res Bull 70:221–227.

Goldstein BL, Klein DN (2014) A review of selected candidate endophenotypes for depression. Clin Psychol Rev 34:417–427.

Gómez-Pinilla F, Dao L, So V (1997) Physical exercise induces FGF-2 and its mRNA in the hippocampus. Brain Res 764:1–8.

González M, Cabrera-Socorro A, Pérez-García CG, Fraser JD, López FJ, Alonso R, Meyer G (2007) Distribution patterns of estrogen receptor α and β in the human cortex and hippocampus during development and adulthood. J Comp Neurol 503:790–802.

Gos T, Schroeter ML, Lessel W, Bernstein HG, Dobrowolny H, Schiltz K, Bogerts B, Steiner J (2013) S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. J Psychiatr Res 47:1694–1699.

Gosselin R-D, Gibney S, O'Malley D, Dinan TG, Cryan JF (2009) Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. Neuroscience 159:915–925.

Gubba EM, Fawcett JW, Herbert J (2004) The effects of corticosterone and dehydroepiandrosterone on neurotrophic factor mRNA expression in primary hippocampal and astrocyte cultures. Mol Brain Res 127:48–59.

Jans LAW, Riedel WJ, Markus CR, Blokland A (2007) Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry 12:522–543.

Jansson L, Wennström M, Johanson A, Tingström A (2009) Glial cell activation in response to electroconvulsive seizures. Prog Neuropsychopharmacol Biol Psychiatry 33:1119–1128.

Jorgensen P, Edgington NP, Schneider BL, Rupeš I, Tyers M, Futcher B (2007) The size of the nucleus increases as yeast cells grow. Mol Biol Cell 18:3523–3532.

Katzenellenbogen BS, Choi I, Delage-Mourroux R, Ediger TR, Martini PG, Montano M, Sun J, Weis K, Katzenellenbogen JA (2000) Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology. J Steroid Biochem Mol Biol 74:279–285.

Kékesi KA, Juhász G, Simor A, Gulyássy P, Szegő ÉM, Hunyadi-Gulyás É, Darula Z, Medzihradszky KF, Palkovitz M, Penke B, Czurkó A (2012) Altered functional protein networks in the prefrontal cortex and amygdala of victims of suicide. PLoS One 7:e50532.

Kempton MJ, Salvador Z, Munafò MR, Geddes JR, Simmons A, Frangou S, Williams SC (2011) Structural neuroimaging studies in major depressive disorder: meta-analysis and comparison with bipolar disorder. Arch Gen Psychiatry 68:675–690.

Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB (1993) Sex and depression in the National Comorbidity Survey I: lifetime prevalence, chronicity and recurrence. J Affect Disord 29:85–96.

Khakh BS, Sofroniew MV (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18:942–952.

Kugaya A, Sanacora G (2005) Beyond monoamines: glutamatergic function in mood disorders. CNS Spectr 10:808–819.

Lenth RV (2006–2009) Java applets for power and sample size [Computer software]. Retrieved 2010 Oct 28 from http://www.stat.uiowa.edu/~rlenth/Power.

Li B, Zhang S, Li M, Hertz L, Peng L (2009) Chronic treatment of astrocytes with therapeutically relevant fluoxetine concentrations enhances cPLA2 expression secondary to 5-HT2B-induced transactivation-mediated ERK1/2 phosphorylation. Psychopharmacology 207:1–12.

Little A (2009) Treatment-resistant depression. Am Fam Physician 80:167–172.

Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC (2009) Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. Eur Neuropsychopharmacol 19:796–805.

Lorenzetti V, Allen NB, Fornito A, Yücel M (2009) Structural brain abnormalities in major depressive disorder: a selective review of recent MRI studies. J Affect Disord 117:1–17.

Lu Y-P, Zeng M, Hu X-Y, Hao X, Swaab DF, Ravid R, Zhou J-N (2003) Estrogen receptor α-immunoreactive astrocytes are increased in the hippocampus in Alzheimer's disease. Exp Neurol 183:482–488.

Machado-Vieira R, Manji HK, Zarate CA (2009) The role of the tripartite glutamatergic synapse in the pathophysiology and therapeutics of mood disorders. Neuroscientist 15:525–539.

Malchow B, Strocka S, Frank F, Bernstein HG, Steiner J, Schneider-Axmann T, Hasan A, Reich-Erkelenz D, Schmitz C, Bogerts B, Falkai P, Schmitt A (2015) Stereological investigation of the posterior hippocampus in affective disorders. J Neural Transm (Vienna) 122:1019–1033.

Mateus-Pinheiro A, Patrício P, Bessa JM, Sousa N, Pinto L (2013) Cell genesis and dendritic plasticity: A neuroplastic pas de deux in the onset and remission from depression. Mol Psychiatry 18:748–750.

McKinnon MC, Yucel K, Nazarov A, MacQueen GM (2009) A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J Psychiatry Neurosci 34:41–54.

Miguel-Hidalgo JJ, Rajkowska G (1999) Immunohistochemistry of neural markers for the study of the laminar architecture in celloidin sections from the human cerebral cortex. J Neurosci Methods 93:69–79.

Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48:861–873.

Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. J Affect Disord 127:230–240.

Müller MB, Lucassen PJ, Yassouridis A, Hoogendijk WJG, Holsboer F, Swaab DF (2001) Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus. Eur J Neurosci 14:1603–1612.

Nagy C, Suderman M, Yang J, Szyf M, Mechawar N, Ernst C, Turecki G (2015) Astrocytic abnormalities and global DNA methylation patterns in depression and suicide. Mol Psychiatry 20:320–328.

Naninck EFG, Lucassen PJ, Bakker J (2011) Sex difference in adolescent depression: do sex hormones determine vulnerability? J Neuroendocrinol 23:383–392.

Nowacka M, Obuchowicz E (2013) BDNF and VEGF in the pathogenesis of stress-induced affective diseases: an insight from experimental studies. Pharmacol Rep 65:535–546.

Österlund MK, Hurd YL (2001) Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. Prog Neurobiol 64:251–267.

Österlund MK (2010) Underlying mechanisms mediating the antidepressant effects of estrogens. Biochim Biophys Acta 1800:1136–1144.

Ott RL, Longnecker M (2001) An introduction to statistical methods and data analysis. 5th ed. Pacific Grove, CA: Duxbury.

Perera TD, Dwork AJ, Keegan KA, Thirumangalakudi L, Lipira CM, Joyce N, Lange C, Higley JD, Rosoklija G, Hen R, Sackeim HA, Coplan JD (2011) Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. PLoS One 6:e17600.

Perez JA, Clinton SM, Turner CA, Watson SJ, Akil H (2009) A new role for FGF2 as an endogenous inhibitor of anxiety. J Neurosci 29:6379–6387.

Popoli M, Yan Z, McEwen BS, Sanacora G (2011) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci 13:22–37.

Quesseveur G, David DJ, Gaillard MC, Pla P, Wu MV, Nguyen HT, Nicolas V, Auregan G, David I, Dranovsky A, Hantraye P, Hen R, Gardier AM, Déglon N, Guiard BP (2013) BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities. Transl Psychiatry 3:e253.

Rajkowska G, Miguel-Hidalgo JJ, Dubey P, Stockmeier CA, Krishnan KR (2005) Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. Biol Psychiatry 58:297–306.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45:1085–1098.

Rajkowska G, Stockmeier CA (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. Curr Drug Targets 14:1225–1236.

Rodrigues SM, LeDoux JE, Sapolsky RM (2009) The influence of stress hormones on fear circuitry. Annu Rev Neurosci 32:289–313.

Samuels BA, Hen R (2011) Neurogenesis and affective disorders. Eur J Neurosci 33:1152–1159.

Sanacora G, Treccani G, Popoli M (2012) Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology 62:63–77.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809.

Schmaal L, Veltman DJ, van Erp TG, Sämann PG, Frodl T, Jahanshad N, Loehrer E, Tiemeier H, Hofman A, Niessen WJ, Vernooij MW, Ikram MA, Wittfeld K, Grabe HJ, Block A, Hegenscheid K, Völzke H, Hoehn D, Czisch M, Lagopoulos J, Hatton SN, Hickie IB, Goya-Maldonado R, Krämer B, Gruber O, Couvy-Duchesne B, Rentería ME, Strike LT, Mills NT, de Zubicaray GI, McMahon KL, Medland SE, Martin NG, Gillespie NA, Wright MJ, Hall GB, MacQueen GM, Frey EM, Carballedo A, van Velzen LS, van Tol MJ, van der Wee NJ, Veer IM, Walter H, Schnell K, Schramm E, Normann C, Schoepf D, Konrad C, Zurowski B, Nickson T, McIntosh AM, Papmeyer M, Whalley HC, Sussmann JE, Godlewska BR, Cowen PJ, Fischer FH, Rose M, Penninx BW, Thompson PM, Hibar DP (2015) Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. http://dx.doi.org/10.1038/mp.2015.69 [Epub ahead of print].

Seeman MV (1997) Psychopathology in women and men: focus on female hormones. Am J Psychiatry 154:1641–1647.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 93:3908–3913.

Si X, Miguel-Hidalgo JJ, O'Dwyer G, Stockmeier CA, Rajkowska G (2004) Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. Neuropsychopharmacology 29:2088–2096.

Stone DJ, Song Y, Anderson CP, Krohn KK, Finch CE, Rozovsky I (1998) Bidirectional transcription regulation of glial fibrillary acidic protein by estradiol in vivo and in vitro. Endocrinology 139: 3202–3209.

Takahashi K, Foster JB, Lin CG (2015) Glutamate transporter EAAT2: regulation, function, and potential as a therapeutic target for neurological and psychiatric disease. Cell Mol Life Sci [Epub ahead of print].

Torres-Platas SG, Nagy C, Wakid M, Turecki G, Mechawar N (2015) Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. Mol Psychiatry. http://dx.doi.org/10.1038/mp.2015.65 [Epub ahead of print].

Walters AD, Bommakanti A, Cohen-Fix O (2012) Shaping the nucleus: factors and forces. J Cell Biochem 113:2813–2821.

Wang Q, Van Heerikhuize J, Aronica E, Kawata M, Seress L, Joels M, Swaab DF, Lucassen PJ (2013) Glucocorticoid receptor protein expression in human hippocampus; stability with age. Neurobiol Aging 34:1662–1673.

Webster M, Witkin LK, Cohen-Fix O (2009) Sizing up the nucleus: Nuclear shape, size and nuclear-envelope assembly. J Cell Sci 122:1477–1486.

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJL, Vos T (2013) Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study. Lancet 382:1575–1586.

Willard SL, Riddle DR, Forbes ME, Shively CA (2013) Cell number and neuropil alterations in subregions of the anterior hippocampus in a female monkey model of depression. Biol Psychiatry 74:890–897.

Ye Y, Wang G, Wang H, Wang X (2011) Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampal of a rat model of depression. Neurosci Lett 503: 15–19.

Young EA, Altemus M (2004) Puberty, ovarian steroids, and stress. Ann N Y Acad Sci 1021:124–133.

Zarate Jr C, Machado-Vieira R, Henter I, Ibrahim L, Diazgranados N, Salvadore G (2010) Glutamatergic modulators: the future of treating mood disorders? Harv Rev Psychiatry 18:293–303.

Zhang H, Zhao Y, Wang Z (2015) Chronic corticosterone exposure reduces hippocampal astrocyte structural plasticity and induces hippocampal atrophy in mice. Neurosci Lett 592:76–81.

Zink M, Vollmayr B, Gebicke-Haerter PJ, Henn FA (2010) Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. Neuropharmacology 58:465–473.

(Accepted 23 December 2015)
(Available online 30 December 2015)