Archival Report

Resilience Is Associated With Larger Dentate Gyrus, While Suicide Decedents With Major Depressive Disorder Have Fewer Granule Neurons

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ABSTRACT

BACKGROUND: Early life adversity (ELA) increases major depressive disorder (MDD) and suicide risk and potentially affects dentate gyrus (DG) plasticity. We reported smaller DG and fewer granular neurons (GNs) in MDD. ELA effects on DG plasticity in suicide decedents with MDD (MDDSui) and resilient subjects (ELA history without MDD or suicide) are unknown.

METHODS: We quantified neural progenitor cells (NPCs), GNs, glia, and DG volume in whole hippocampus post-mortem in four groups of drug-free, neuropathology-free subjects (N = 52 total): psychological autopsy-defined MDDSui and control subjects with and without ELA (before 15 years of age).

RESULTS: ELA was associated with larger DG ($p < .0001$) and trending fewer NPCs ($p = .0190$) only in control subjects in whole DG, showing no effect on NPCs and DG volume in MDDSui. ELA exposure was associated with more GNs ($p = .0003$) and a trend for more glia ($p = .0160$) in whole DG in MDDSui and control subjects. MDDSui without ELA had fewer anterior and mid DG GNs ($p < .0001$), fewer anterior DG NPCs ($p < .0001$), and smaller whole DG volume ($p = .0005$) compared with control subjects without ELA. In MDDSui, lower Global Assessment Scale score correlated with fewer GNs and smaller DG.

CONCLUSIONS: Resilience to ELA involves a larger DG, perhaps related to more neurogenesis depleting NPCs, and because mature GNs and glia numbers do not differ in the resilient group, perhaps there are effects on process extension and synaptic load that can be examined in future studies. In MDDSui without ELA, smaller DG volume, with fewer GNs and NPCs, suggests less neurogenesis and/or more apoptosis and dendrite changes.

Keywords: Granule cell, Major depression, Neural progenitors, Postmortem, Stress, Suicide

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Early life adversity (ELA) may increase the risk of major depressive disorder (MDD) and suicidal behavior during adulthood (1), according to a twin study (2) and in patients with various psychiatric disorders (3,4). Exposure to childhood physical or sexual abuse or witnessing domestic violence could explain 16% to 50% of suicidal ideation and attempts (5). A moderate level of heritability has been identified in MDD (6) and, separately, suicide (7). The role of a gene-by-environment interaction is not so clear-cut in MDD (8), and MDD seems to be associated with childhood trauma and polygenic risk, without an interaction effect between the two (9).

Decreased glucocorticoid receptor expression in postmortem hippocampus of suicide decedents with ELA versus suicides without ELA was linked to DNA methylation of the glucocorticoid receptor gene promoter (10). In animal studies, ELA affects the brain and behaviors related to human suicide such as mood and aggression. Infant primates exposed to maternal separation later exhibit altered social behavior, neurochemistry, and hypothalamic-pituitary-adrenal axis reactivity (11). In rodents, maternal deprivation alters hippocampus cellular plasticity (12), neurogenesis, and cell survival (13), and it resets the hypothalamic-pituitary-adrenal axis by reduced expression of glucocorticoid receptor and overexpression of the mineralocorticoid receptor (14).

We reported smaller postmortem rostral (anterior–mid) dentate gyrus (DG) and fewer granule neurons (GNs) in untreated MDD suicide decedents without changes in DG glial cells (15). Similarly, smaller postmortem rostral hippocampus was described in a macaque depression model (16). Brain imaging findings show smaller hippocampus and amygdala in humans with reported ELA (17) and hippocampal volume-mediating effects of ELA on behavioral abnormalities (18). Smaller mid hippocampus DG and cornu ammonis were found in unmedicated MDD (19) and posttraumatic stress disorder (20). Interestingly, the rodent ventral DG (corresponding to the anterior–mid primate DG) regulates stress responses (21,22). Because not all

individuals exposed to ELA develop psychopathologies such as MDD and suicide, outcome of ELA may depend on neurobiological adaptations following or preceding the trauma (23).

Hippocampus (19,20,24–29) and particularly DG (15,30) are stress-responsive regions affected by trauma and are abnormal in MDD and suicide. Despite the described anatomical, neurochemical, and hormonal changes associated with stress exposure and MDD or suicide, a paucity of information exists on how ELA affects DG neuroplasticity and volume in subjects with MDD who died by suicide (MDDSui) versus resilient subjects who, despite ELA exposure, did not develop lifetime psychopathology and did not die from suicide. To address this question, in age- and sex-matched MDDSui and nonpsychiatric, nonsuicide control subjects with and without reported ELA exposure, we characterized and quantified nestin-immunoreactive (IR) neural progenitor cells (NPCs), mature GNs IR for neuronal nuclear marker, glia (morphologically identified by Nissl stain), and DG volume in anterior, mid, and posterior hippocampus. In smaller samples, we showed no significant differences in number of nestin-IR NPCs between control subjects and subjects with mood disorder (MDD or bipolar disorder), which included suicide and nonsuicide decedents (31,32). Nestin has been extensively used in mice as marker of NPCs (33). Transgenic *Nestin-Cre* mouse lines have been broadly utilized to trace the neuronal differentiation of neural stem cells (34). Doublecortin was not used in this study because, in contrast to nestin, it is sensitive to breakdown during postmortem delay (35).

More than half of the subjects in this study were never studied before, and here we assessed ELA exposure effects in MDDSui and control subjects. We examined anterior, mid, and posterior DG volumes separately because subregional differences were reported using magnetic resonance imaging in MDD (19,25) and with stress exposure (28,36).

METHODS AND MATERIALS

Subjects

We selected 52 subjects (Tables 1 and 2): age- and sex-matched control subjects with ELA (Control-wELA), control subjects without ELA (Control-w/oELA), MDDSui with ELA (MDDSui-wELA), and MDD without ELA (MDDSui-w/oELA) (n = 13/group) who died suddenly without prolonged agonal phase. Subjects were clinically characterized by our validated psychological autopsy (37). The assessment of adversity was done using a checklist of questions covering deprivation events, including death or divorce of parents, growing up in foster care or an orphanage, and active physical or sexual abuse. We confined our analyses to events occurring before 15 years of age. Clinical evaluation included the Global Assessment Scale (GAS) to assess DSM-IV Axis V global functioning (38) shortly before death.

Subjects had clear neuropathology (no microvascular disease, plaques, or evidence of brain pathology or damage) and clear brain and blood toxicology for psychotropic medications and substances. We excluded subjects with psychotropic drug prescriptions within 3 months of death. History of more remote medication use was not available. We excluded subjects with obstetrical complications at birth, history of alcohol or drug abuse, or positive blood alcohol level at death. Nicotine use

was not excluded, and we analyzed the distribution of people who were smokers in each group and the effect of smoking on GN, glial cell, and NPC numbers and DG volume.

Brain Collection and Tissue Processing

Tissue was obtained from the Macedonian/New York State Psychiatric Institute Brain Collection with institutional review board approval. Approximately half of the subjects and control subjects were from the United States and half were from Macedonia, and they included individuals from Caucasian, Hispanic, and African descent (Table 1). Cerebellar tissue was used for brain pH determination and toxicology (32), excluding tissue that had pH < 5.68. Postmortem interval (PMI) may affect brain proteins (39,40) and was limited to 24 hours. The hippocampal formation was dissected from frozen coronal blocks, fixed in 4% paraformaldehyde at 4°C, cryoprotected in 30% sucrose, sectioned at 50 μm on a sliding microtome (Microm HM450, ThermoFisher Scientific, Waltham, MA), and stored in 40-well boxes at −20°C in cryoprotectant. One section every 500 μm was stained for Nissl (Supplemental Figure S1) and used for anatomical alignment along the DG rostrocaudal axis of sections processed for immunocytochemistry. In our hands, fixation time and solutions affect tissue immunoreactivity for nestin and neuronal nuclear marker, and suboptimal perfusion of mice brain also impairs immunohistochemistry results. Therefore, results obtained in tissue fixed with different fixatives or for different amounts of time are not comparable (41,42).

Immunohistochemistry

Immunohistochemistry was performed on sections at 2-mm intervals as reported (43). We used our modified immunocytochemistry method (neuronal nuclear marker mouse monoclonal antibody, 1:100,000; Chemicon, Temecula, CA) to make all GNs countable and not overly packed (15), and Nissl stain was used to identify glial cells (Figure 1 and Supplemental Figure S1). To identify NPCs, we used anti-nestin mouse monoclonal antibody (1:8000; Chemicon) (Figure 2).

Stereology

Immunoassayed sections at 2-mm intervals throughout the whole anterior–posterior extent of DG were sampled using Stereo Investigator software (MBF Bioscience, Williston, VT) as reported (43). The optical disector with fractionator method (44) was used to estimate total cell number (N) using the formula

$$N = \sum Q^- \times \left(\frac{t}{h}\right) \times \left(\frac{1}{asf}\right) \times \left(\frac{1}{ssf}\right),$$ where $\sum Q^-$ is the

number of cells counted, t is mean section thickness, h is optical disector height, asf is sampling fraction area (counting frame area/x,y step area), and ssf is section sampling fraction (1/section interval). Sampling parameters were $asf = 0.02$, $ssf = 0.05$, grid size = 210 × 210 μm, and counting frame = 30 × 30 μm. Average $\sum Q^-$ was 672 in anterior DG, 367 in mid DG, and 424 in posterior DG. We used the optical fractionator based on the number-weighted mean section thickness to avoid shrinkage-based bias. We applied oversampling for nestin-IR NPCs, as is required when estimating the number of rare elements. Guard zones 3 μm above and below the disector were used, ensuring that they were sufficient to exclude damaged cells and tissue that may bias the count. Zooming

Resiliency-Related Human Hippocampus Neuroplasticity

Table 1. Demographic and Biological Characteristics of Subjects

Variables	Control-w/oELA (n = 13) Mean (SD) [Min, Max]	Control-wELA (n = 13) Mean (SD) [Min, Max]	MDDSui-w/oELA (n = 13) Mean (SD) [Min, Max]	MDDSui-w/ELA (n = 13) Mean (SD) [Min, Max]	Statistics $F_{3,51}$	ANOVA p Value
Age, Years	38.5 (14.5) [14-58]	36.6 (18.5) [18-66]	41.5 (10.9) [25-61]	36.3 (20.1) [13-65]	0.267	.849
PMI	15.3 (5.1) [6.00-22.0]	14.2 (4.0) [8.00-21.00]	14.7 (6.8) [3.00-24.00]	17.5 (6.4) [3.00-27.00]	2.095	.113
Brain pH	6.4 (0.4) [5.68-6.87]	6.5 (0.3) [5.93-6.84]	6.5 (0.3) [6.00-6.93]	6.4 (0.3) [6.20-6.77]	0.826	.488
Brain Weight, g	1399.2 (124.6) [1213-1600]	1321.2 (188.6) [1000-1450]	1369.5 (211.5) [1120-1550]	1475.0 (91.9) [1410-1540]	0.573	.640
BMI	26.3 (5.5) [19.57-35.70]	25.5 (6.6) [19.98-42.66]	24.5 (4.6) [20.36-31.35]	22.2 (2.7) [19.53-26.66]	0.608	.617
	n (%)	n (%)	n (%)	n (%)	χ^2 Test	χ^2 p Value
Sex					3.611	.307
Male	7 (53.8)	11 (84.6)	8 (61.5)	10 (76.9)		
Female	6 (46.2)	2 (15.4)	5 (38.5)	3 (23.1)		
Ethnicity					10.292	.113
Caucasian descent	12 (92.3)	8 (61.5)	12 (92.3)	10 (76.9)		
African descent	1 (7.7)	3 (23.1)	0	1 (7.7)		
Hispanic descent	0	2 (15.4)	1 (7.7)	2 (15.4)		
Cause of Death						
Blunt trauma	8	2	–	–		
Cardiovascular	3	9	–	–		
Respiratory	2	–	–	–		
Hemorrhage	–	2	–	–		
Hanging/drowning	–	–	9	6		
Overdose/poisoning	–	–	3	4		
Immolation	–	–	1	–		
Fall from height	–	–	–	3		
Brain/Blood Toxicology						
ACE inhibitors	0	1	1	1		
Lidocaine/opioids	4	2	1	0		
None	9	10	11	12		

ACE, angiotensin-converting enzyme; ANOVA, analysis of variance; BMI, body mass index; Control-wELA, resilient control subjects with ELA; Control-w/oELA, control subjects without ELA; ELA, early life adversity; MDD, major depressive disorder; MDDSui-wELA, subjects with MDD who died by suicide with ELA; MDDSui-w/oELA, subjects with MDD who died by suicide without ELA; PMI, postmortem interval.

Table 2. Subject Sample Clinical Features

Variables	Control-w/oELA (a) (n = 13) Mean (SD) [Min, Max]	Control-wELA (b) (n = 13) Mean (SD) [Min, Max]	MDDSui-w/oELA (c) (n = 13) Mean (SD) [Min, Max]	MDDSui-w/ELA (d) (n = 13) Mean (SD) [Min, Max]	$F_{3,51}$	ANOVA p Value	Tukey Post Hoc Test
Age of MDD Onset, Years	–	–	35.7 (14.2) [17-59]	41.1 (18.1) [17-64]	0.531	.476	
Duration of MDD, Years	–	–	6.1 (7.2) [0.3-20.00]	3.6 (4.3) [0.3-11.00]	0.780	.389	
Number of MDEs in Lifetime	–	–	2.0 (1.5) [1-5]	1.2 (0.4) [1-2]	1.726	.212	
GAS Score	87.7 (7.1) [76-100]	85.6 (9.1) [65-100]	39.0 (16.1) [1-55]	41.8 (25.7) [1-75]	24.573	.000	a>c, a>d, b>c, b>d: p < .001
BGAS Score	12.5 (3.6) [10-21]	13.7 (6.7) [10-30]	12.3 (2.9) [10-18]	14.2 (4.7) [10-23]	0.433	.731	
SPRS Total Score	9.8 (3.2) [6.00-17.00]	9.0 (2.6) [6.00-13.00]	12.9 (2.8) [10.00-17.00]	14.8 (3.7) [10.00-21.00]	7.187	.001	a>d: p = .005 b>c: p = .031 b>d: p = .002
SPRS Axis IV Impact Score	1.8 (1.0) [1.00-4.00]	2.2 (1.0) [1.00-4.00]	4.1 (1.3) [2.00-6.00]	4.3 (1.0) [2.00-5.00]	14.108	.000	a>c, a>d: p < .001 b>c, b>d: p = .002
	n (%)	n (%)	n (%)	n (%)	χ^2 Test	χ^2 p Value	
SPRS Axis IV Impact Score					29.497	.014	
None-minimal (1-2)	10 (76.9)	8 (61.5)	2 (15.4)	2 (15.4)			
Mild-moderate (3-4)	3 (23.1)	5 (38.5)	4 (30.8)	3 (23.1)			
Severe-extreme (5-6)	0	0	7 (53.8)	8 (61.5)			

ANOVA, analysis of variance; BGAS, Brown–Goodwin Aggression Scale; Control-wELA, resilient control subjects with ELA; Control-w/oELA, control subjects without ELA; ELA, early life adversity; GAS, Global Assessment Scale; MDD, major depressive disorder; MDDSui-wELA, subjects with MDD who died by suicide with ELA; MDDSui-w/oELA, subjects with MDD who died by suicide without ELA; MDE, major depressive episode; SPRS, St. Paul–Ramsey Scale.
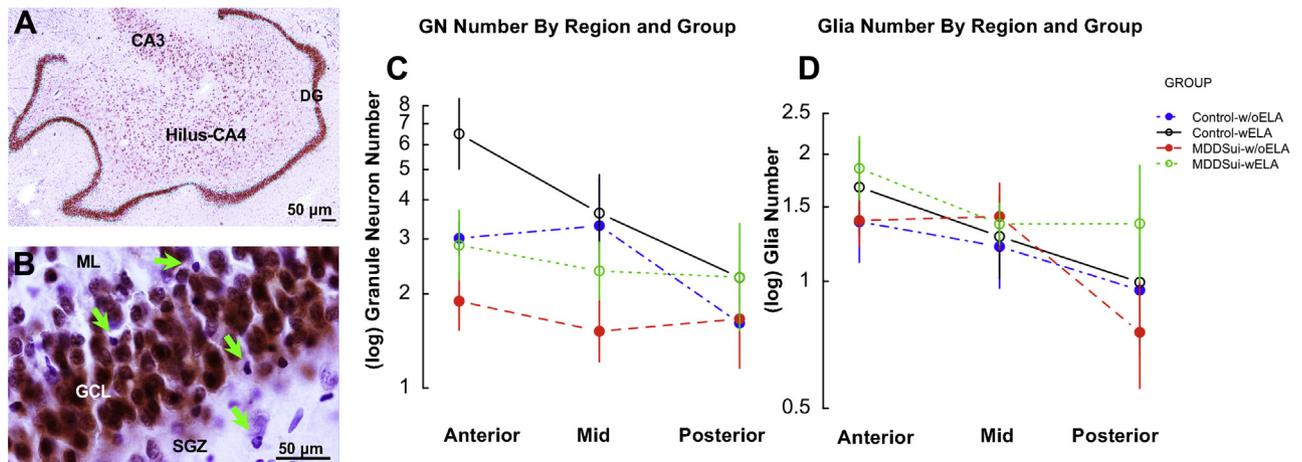
Figure 1. Mature granule neurons (GNs) in the human hippocampus from subjects with and without reported early life adversity (ELA) exposure. **(A)** Neuronal nuclear marker (NeuN)-immunoreactive granule neurons (in brown) were found in the dentate gyrus (DG) and in the hilus and cornu ammonis (CA) regions. The blue-green outline defines the granule cell layer (GCL) region of interest for counting granule neurons with stereology. **(B)** NeuN-immunoreactive granule neurons (in brown) were packed within the GCL, and less dense in the molecular layer (ML) and subgranular zone (SGZ), and had their characteristic round shape, readily distinguishable from pyramidal neurons in the hilus. Glial cells in the DG were stained for Nissl with cresyl violet and were NeuN negative and identified for showing heterochromatin clumps, sparse cytoplasm, and smaller cell body size than GNs (115) (green arrows). We did not differentiate morphologically between oligodendrocytes, with their compact, darker-stained nuclei, and astrocytes and microglia, which have larger, lighter-stained nuclei with more granular chromatin (116). Few thousands of NeuN-negative and Nissl-stained cells might be neural progenitors, or young neurons, although glial cells are on the order of thousands and few thousands of glial cells would not change the results. Endothelial cells have a distinguishable elongated morphology and were not counted. Sampling of GNs and glial cells, which are very packed, was performed with a 40× air objective (numerical aperture: 0.95), which provided the same results as the tested sampling with a 60× oil objective (numerical aperture: 1.42). **(C)** Numbers of GNs in anterior, mid, and posterior DG in control subjects without ELA (Control-w/oELA), resilient control subjects with ELA (Control-wELA), and subjects with major depressive disorder (MDD) who died by suicide (MDDSui) with ELA (MDDSui-wELA) and without ELA (MDDSui-w/oELA). **(D)** Numbers of glial cells in anterior, mid, and posterior DG in Control-w/oELA, Control-wELA, MDDSui-wELA, and MDDSui-w/oELA. MDDSui had fewer anterior–mid DG GNs ($p < .0001$) and ELA exposure associated with more GNs ($p = .0003$) in both groups and all DG regions. ELA was associated with more glia in both groups ($p = .0160$) in all regions. Outcome value labels are shown on the original scale, although mean and SE were calculated on the log-transformed data.

into the tissue, we verified that the immunostaining penetrated throughout the whole section thickness. Average section thickness after fixation and processing was similar among groups (Control-wELA: 22.1 ± 1.2; Control-w/oELA: 21.8 ± 1.6; MDDSui-wELA: 21.6 ± 0.9; MDDSui-w/oELA: 23.6 ± 2.7), $F_{3,47} = 41.242$, $p = .35$. We limited the coefficient of error to below 10% for each type of cell counted.

Statistical Analysis

We used SPSS (version 24 for Mac; IBM Corp., Armonk, NY) and the statistical language R (version 3.1.2; R Foundation for Statistical Computing, Vienna, Austria). All quantitative measures were graphically inspected by group for normality, outliers, and inconsistent values. Some data were missing for the posterior region of DG; in total, 7 subjects (13%) lacked GN and NPC number, 6 subjects (12%) lacked glial cell number, and 4 subjects (8%) lacked DG volume, while in the mid region of DG, 8 subjects (15%) lacked glial cell number. Because the main analyses included all brain regions, no subject was excluded because of missing values. Outcome measures with skewed distributions and several outliers that were log transformed were GN, NPC, and glial cell numbers; any remaining outliers were winsorized, that is, censored or replaced by the nearest nonoutlier value. This approach included all subjects with data and eliminated the need to repeat analyses without the outliers, a problematic solution in the case of low group sizes.

Four observations were winsorized (Supplemental Table S1) for NPC numbers (two from the posterior region, one from the mid region, and one from the anterior region), five observations were winsorized for glial cell numbers (all from the posterior region), and 12 observations were winsorized for DG volume (eight from the posterior region, three from the mid region, and one from the anterior region).

Two-way analysis of variance was used to test for between-group differences in age, PMI, brain weight, pH, and GAS score, with Tukey's honestly significant difference test to correct for multiple comparisons. Data were expressed as mean ± SD. Chi-square tested group differences in sex distribution. Regression analyses tested correlations among cell numbers, DG volume, and demographic and clinical variables.

The four outcome measures—GN number, glial cell number, NPC number, and DG volume—were analyzed using separate mixed-effect models (45), with two between-subject factors (group and ELA), one within-subject factor (DG region with anterior, mid, and posterior as levels), all possible two-way interactions of these three factors as fixed effects, and subject-specific random intercepts to allow for within-subject correlation. We used the following formula:

$$\text{Outcome} \sim \text{ELA} + \text{MDDSuicide} + \text{Region} + \text{ELA} \times \text{MDDSuicide} + \text{MDDSuicide} \times \text{Region} + \text{ELA} \times \text{Region}$$
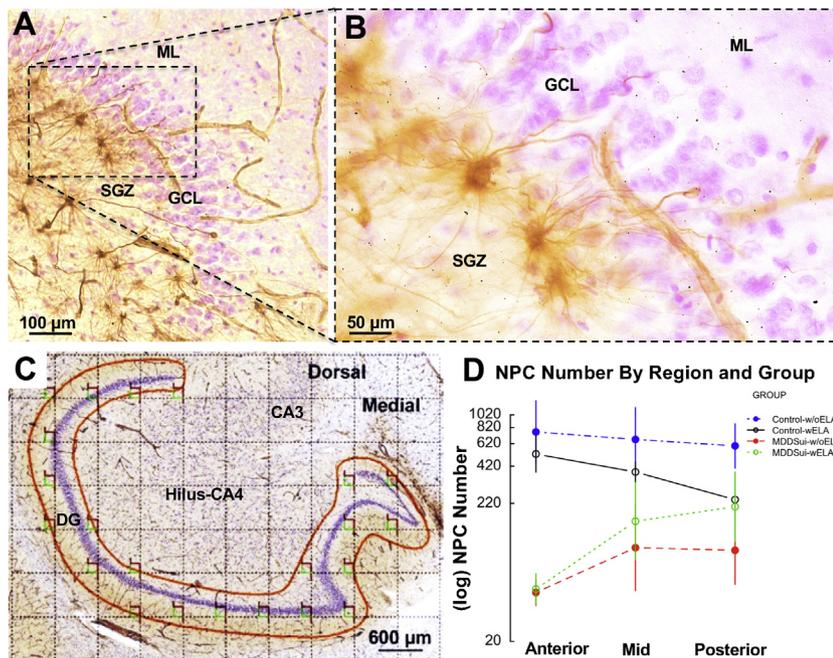
Figure 2. Neural progenitor cells (NPCs) in the dentate gyrus (DG) from subjects with major depressive disorder (MDD) and control subjects with and without reported early life adversity (ELA). **(A)** Multipolar NPCs with multiple dendritic processes and remodeling nestin-immunoreactive (IR) capillaries in the subgranular zone (SGZ). Granular neurons and glia are stained for Nissl with cresyl violet. NPCs, showing nestin-IR cell bodies and processes, were found in the SGZ, but not in the granular cell layer (GCL) and molecular layer (ML), and displayed processes in contact with remodeling capillaries, which are also nestin IR, have a lumen, and are quite distinguishable from NPCs, as previously described (32). **(B)** Enlargement of the rectangle in **(A)**. NPC dendrites touched the capillary and extended into the GCL. Nestin-IR blood vessels displayed their characteristic tubular appearance. **(C)** The GCL, ML, and SGZ are outlined (red line) to define the DG, where nestin-IR NPCs (in brown) were counted. Sampling of NPCs was performed with a 60× oil objective (numerical aperture: 1.42). **(D)** Numbers of NPCs in anterior, mid, and posterior DG in control subjects without ELA (Control-w/oELA), resilient control subjects with ELA (Control-wELA), and subjects with MDD who died by suicide (MDDSui) with ELA (MDDSui-wELA) and without ELA (MDDSui-w/oELA). MDDSui had fewer NPCs than control subjects in anterior DG ($p <$ .0001). ELA affected NPCs differently in MDDSui and control subjects in all regions ($p =$ .0059), with fewer NPCs observed only in control subjects ($p =$ .0190). Outcome value labels are shown on the original scale, although mean and SE were calculated on the log-transformed data. CA, cornu ammonis.

where MDDSuicide is major depression–suicide. Interactions between each of the two main predictors with brain region allow for region-specific effects of ELA or suicide to be identified. Nonsignificant interactions, but not main effects, were then removed in a stepwise fashion from each model, and the final models were reported (Table 3). We used a Bonferroni-corrected experiment-wise error rate of 0.0125 for significance cutoff to adjust for multiple testing.

The suicide rate is four times higher in male individuals (46), and we matched the control subjects to the suicide decedent group's demographics. Mixed-effect regression models were fit, for each outcome separately, in non–ELA subjects only, with sex and brain region as the predictors, with and without adding suicide status as covariate, and subject-specific random intercepts.

RESULTS

Effects of Demographic and Other Biological Variables

There were no between-group differences in demographic variables (Table 1). Regression analysis did not show a significant correlation between age, brain weight, pH, or PMI and NPC, GN, or glial cell numbers or with DG volume in anterior, mid, and posterior DG ($p >$ .05). Sex had no significant effect in any of the mixed- effect regression models (all ps $>$ .05), although we need to point out that there were fewer women than men and the number of women in the ELA-exposed groups was particularly low (3 suicides and 1 control subject).

Smokers were more prevalent among people who died by suicide ($\chi^2_1 =$ 8.70, $p =$ .0032), and there was no difference in

the frequency of smokers between ELA-exposed and nonexposed individuals ($\chi^2_1 =$ 2.48, $p =$ .1153). After adjusting for smoking status, DG volume, and NPC count, group differences remained significant, while smoking had no significant effect on DG volume ($p =$ .6430) or on NPC number ($p =$ .8811). Therefore, we did not include smoking status in any of the final models.

Effects of MDDSui and Adversity

ELA exposure was associated with more GN ($b =$ 0.39, $p =$ .0003) in all regions in MDDSui and control subjects because interactions between ELA and DG region and between ELA and group were not significant (Figure 1C and Supplemental Figure S2A). There was a group effect that differed by region (region by group interaction: $F_{2,93} =$ 9.28, $p =$ .0002) on (log-transformed) number of GNs; MDDSui had fewer neurons versus controls in anterior DG ($b = -$0.64, $p <$ .0001) and mid DG (which did not differ from the effect in anterior DG, $p =$ .8072), and the posterior DG group effect was smaller versus anterior DG (posterior DG by group interaction: $b =$ 0.67, $p =$ .0002) (Table 3 and Figure 1C).

There was a trend association of ELA with more glia in all regions and in both MDDSui and control subjects ($b =$ 0.19, $p =$ .0160, not significant at the multiple testing–adjusted .0125 level); there were fewer glial cells in posterior DG versus anterior DG regardless of group ($b = -$0.45, $p <$ .0001) (Table 3, Figure 1D, and Supplemental Figure S2B).

NPC number (log-transformed) changes were driven by a group by adversity interaction ($F_{1,48} =$ 8.31, $p =$ .0059) and a group by DG region interaction ($F_{2,93} =$ 12.78, $p <$ .0001). There was a trend toward fewer NPCs associated with ELA

Table 3. Effects of MDDSuicide, Early Life Adversity, and Dentate Gyrus Region (Anterior, Mid, or Posterior) on Dentate Gyrus Cells and Volume

	B	SE	df	t Value	p Value
(log) Granule Neuron Number ~ ELA + MDDSuicide + Region + MDDSuicide × Region					
MDDSuicide	−0.64	0.14	49	−4.67	< .0001[a]
ELA	0.39	0.10	49	3.86	.0003[a]
Region (MidDG vs. AntDG)	−0.25	0.12	93	−2.13	.0362
Region (PostDG vs. AntDG)	−0.85	0.12	93	−6.97	< .001[a]
Region (MidDG vs. AntDG) × MDDSuicide[b]	0.04	0.16	93	0.24	.8072
Region (PostDG vs. AntDG) × MDDSuicide[b]	0.67	0.17	93	3.90	.0002[a]
(log) Glia Number ~ ELA + MDDSuicide + Region					
MDDSuicide	0.06	0.08	49	0.81	.4225
ELA	0.19	0.08	49	2.49	.0160
Region (MidDG vs. AntDG)	−0.16	0.09	88	−1.76	.0825
Region (PostDG vs. AntDG)	−0.45	0.09	88	−4.91	< .0001[a]
(log) Neural Progenitor Cell Number ~ ELA + MDDSuicide + Region + ELA × MDDSuicide + MDDSuicide × Region					
MDDSuicide	−3.06	0.30	48	−10.13	< .0001[a]
ELA	−0.60	0.25	48	−2.43	.0190
Region (MidDG vs. AntDG)	−0.22	0.22	93	−1.01	.3148
Region (PostDG vs. AntDG)	−0.48	0.23	93	−2.08	.0399
Region (MidDG vs. AntDG) × MDDSuicide[b]	1.20	0.31	93	3.87	.0002[a]
Region (PostDG vs. AntDG) × MDDSuicide[b]	1.56	0.32	93	4.81	< .0001[a]
MDDSuicide × ELA	1.00	0.35	48	2.88	.0059[a]
Dentate Gyrus Volume ~ ELA + MDDSuicide + Region + ELA × MDDSuicide					
MDDSuicide	−10.51	2.82	48	−3.73	.0005[a]
ELA	18.76	2.82	48	6.66	< .0001[a]
Region (MidDG vs. AntDG)	−7.18	2.42	98	−2.96	.0039[a]
Region (PostDG vs. AntDG)	−4.11	2.47	98	−1.66	.0999
MDDSuicide × ELA[c]	−21.09	4.01	48	−5.26	< .0001[a]

AntDG, anterior dentate gyrus; ELA, early life adversity; MDD, major depressive disorder; MDDSuicide, subjects with MDD who died by suicide; MidDG, mid dentate gyrus; PostDG, posterior dentate gyrus.

[a]These p values are significant at the α = .0125 level (i.e., adjusted for the four outcomes using the Bonferroni method).

[b]When an interaction between MDDSuicide and Region is included in the model, the main effect of MDDSuicide denotes the test of difference between suicides and control subjects in the AntDG, while the interaction terms test the difference in MDDSuicide effect between MidDG or PostDG and AntDG.

[c]When an interaction between MDDSuicide and ELA is included in the model, the main effect of ELA tests the difference between control subjects with and without ELA, the main effect of MDDSuicide tests the difference between suicide decedents with MDD without ELA and control subjects without ELA, while the interaction term tests the difference in the ELA effect between suicide decedents with MDD and control subjects.

exposure only in control subjects (b = 0.60, p = .0190, not significant at the multiple testing–adjusted .0125 level), contrasting with no difference between MDDSui-w/oELA and MDDSui-wELA in any DG regions (Table 3, Figure 2D, and Supplemental Figure S3A).

Breaking down the group by DG region interaction further, fewer NPCs were found in MDDSui-w/oELA versus Control-w/oELA in anterior DG only (b = −3.06, p < .0001), while between-group differences were smaller in mid and posterior DG, reflected in a region by diagnostic group interaction in mid DG (b = 1.20, p = .0002) and posterior DG (b = 1.56, p < .0001) (Table 3 and Figure 2D).

For DG volume, there was an ELA by diagnostic group interaction (p < .0001) such that Control-wELA had larger DG than Control-w/oELA (b = 18.76, p < .0001) in all DG regions.

MDDSui-w/oELA had smaller DG volume than Control-w/oELA (b = −10.51, p = .0005), while the difference in volume for MDDSui-wELA and MDDSui-w/oELA was smaller owing to a smaller volume for MDDSui-wELA than what would have

been expected from an additive model (interaction: b = −21.09, p < .0001) (Table 3, Figure 3D, and Supplemental Figure S3).

There was no significant region by ELA or region by diagnostic group interaction, meaning that suicide and adversity differences did not differ by region.

GAS score shortly prior to death confirmed better functioning of control subjects versus MDDSui (p < .0001) (Table 2). Better GAS score correlated with more anterior DG GNs and larger volume (Figure 4). There was no correlation between GAS score and GN number in mid and posterior DG or with NPC number in any region (not shown).

DISCUSSION

This is the first postmortem study to assess ELA effects on DG cell numbers and volume in MDDSui and nonpsychiatric, nonsuicide subjects. An index of the impact of ELA would be a biological effect in control subjects or MDDSui exposed to ELA
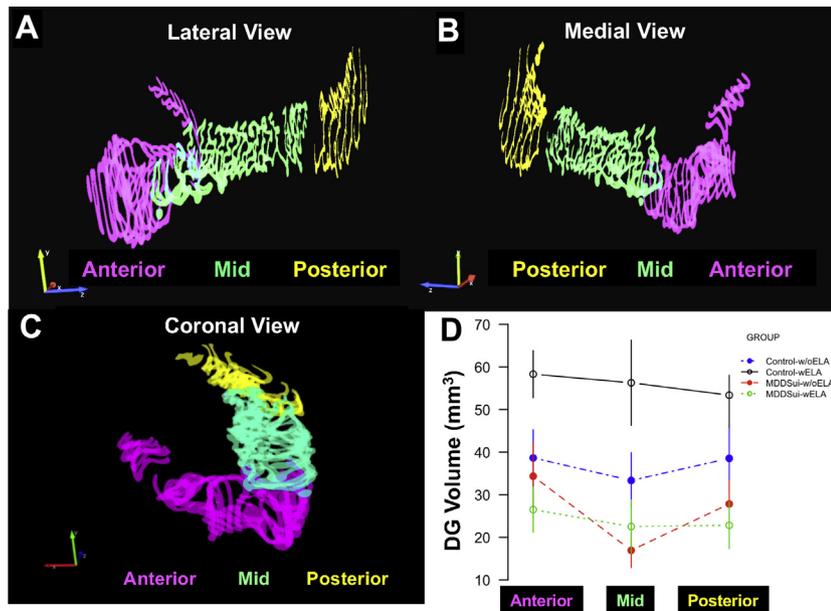
Figure 3. Dentate gyrus (DG) volume in subjects with and without reported early life adversity (ELA) exposure. **(A–C)** Lateral **(A)**, medial **(B)**, and coronal **(C)** views of DG outlines, including granule cell layer, molecular layer, and subgranular zone, traced with stereology software and aligned from the anterior to the posterior extent of the hippocampus. Anterior, mid, and posterior DG volumes were calculated including molecular layer, granule cell layer, and subgranular zone, as previously described (43). **(D)** DG volume in anterior, mid, and posterior hippocampus in control subjects without ELA (Control-w/oELA), resilient control subjects with ELA (Control-wELA), subjects with major depressive disorder (MDD) who died by suicide (MDDSui) with ELA (MDDSui-wELA) and without ELA (MDDSui-w/oELA). MDDSui had smaller DG (*p* = .0005) than controls, and ELA was associated with larger DG in control subjects (*p* < .0001) but not in MDDSui.

versus nonexposed subjects of the same group. An index of resilience is an effect seen in ELA-exposed control subjects versus ELA-exposed MDDSui. We found larger DG with ELA only in control subjects, indicating a resilience effect. ELA exposure was also associated with fewer NPCs only in control subjects. In contrast, ELA was associated with more GN in both MDDSui and control subjects in all DG regions, a finding not consistent with either vulnerability or resilience (Supplemental Figure S4).

ELA association with more GNs in whole DG suggests that GNs may mediate ELA-related consequences for memory and emotional responses, functions depending on posterior and anterior hippocampus, respectively (47).

Fewer GNs in anterior and mid DG in MDDSui versus control subjects agrees with our previous findings (15). Anterior DG, implicated in emotional regulation (48), is connected to amygdala–prefrontal cortex in primates (49). Amygdala is involved in stress and fear responses (50), and prefrontal cortex, anterior cingulate, and reward pathways are involved in decision making (51,52). These brain regions can be

anatomically or physiologically affected in MDD and suicidal behavior (53), where problem solving (54) and cognitive flexibility (55) are reportedly impaired.

Opposite effects of ELA on NPCs in control subjects and MDDSui, with a trend toward fewer NPCs only in Control-wELA versus Control-w/oELA, may suggest that the NPC pool got more exhausted in control subjects earlier in life in response to ELA exposure, possibly to generate more GNs, compared with what happens in MDDSui. If there is a finite quiescent neural progenitor pool (56) or, alternatively, if nestin-expressing radial glia-like precursors, after several self-renewing divisions, undergo glial differentiation (33), subjects generating more GNs for stress adaptation would exhaust their progenitor pool earlier in life (57), as observed in control subjects in anterior DG and with ELA exposure throughout whole DG. Another scenario could be that Control-wELA, who have more GNs, have blunted NPC proliferation later in life as a consequence of having more GNs. These possibilities can be tested in future mouse studies.
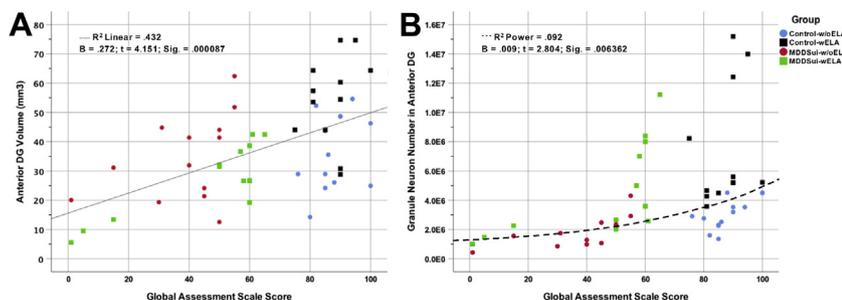

Figure 4. Global functioning score (Global Assessment Scale [GAS]) correlated with anterior dentate gyrus (DG) granule neuron number and DG volume. **(A)** Better GAS score correlated with larger anterior DG volume. **(B)** Better GAS score correlated with more granule neurons in anterior DG. No other correlations between GAS score and DG cell numbers and DG volume were found in any DG subregion (not shown). GAS score showed no correlation with glial cell numbers (not shown). Control-wELA, resilient control subjects with early life adversity; Control-w/oELA, control subjects without ELA; MDDSui-wELA, subjects with major depressive disorder who died by suicide with ELA; MDDSui-w/oELA, subjects with MDD who died by suicide without ELA.

Fewer anterior DG NPCs in MDDSui-w/oELA versus Control-w/oELA suggests possibly less efficient NPC proliferation in MDDSui, leading to fewer GNs, perhaps related to the neurobiology of MDDSui and affecting emotional regulation, which depends on anterior DG (48). Non-ELA-exposed MDDSui had the lowest numbers of NPCs and GNs, suggesting impaired NPC proliferation and maturation, possibly throughout their lifetime and independently of ELA. In line with this hypothesis, mice showing reduced sucrose preference following exposure to chronic unpredictable stress have decreased neurogenesis (58). Social isolation produced memory deficits and aggression in mice and blunted neuronal differentiation (59). Nevertheless, these studies did not dissect which stage of neurogenesis is impaired (proliferation or maturation at different steps of the neuronal differentiation cascade) or whether there is a prevalent effect of increased apoptosis of NPCs or GNs. Moreover, animal depression models obtained through stress or corticosteroid exposure do not allow dissecting effects related to stress, MDD, or suicide. In a previous study with a smaller sample of suicide and nonsuicide MDD subjects, we did not detect differences from control subjects in nestin-IR NPCs (32,60). It remains to be determined whether the NPC deficit is related mostly to suicide or MDD.

Stress exposure may facilitate differentiation of NPCs toward the glial fate rather than the neuronal fate (61), in line with our findings of a trend toward more glia in all regions with ELA exposure in both MDDSui and control subjects. A relationship between hippocampal microglia and neurogenesis has been reported in a mouse chronic unpredictable stress model (62). Deleting the microglial fractalkine receptor gene involved in microglia–neuron crosstalk resulted in resiliency to stress and normal neurogenesis (58).

An absence of glial cell number changes in MDDSui suggests a primary role of GNs in the pathophysiology of MDD and suicide. Nevertheless, lower glial density in neocortex in bipolar disorder (63), less glia in amygdala in MDD (64), and lower astrocyte density in left DG in women and in hippocampus hilus in both sexes in MDD (65) were reported. Changes in different glial cell subpopulations could not be ruled out because we did not examine separately numbers of astrocytes, oligodendrocytes, and microglia.

Larger volume in all DG regions only in ELA-exposed control subjects may represent a resiliency effect and suggests either that MDDSui had a negative neurobiological response to ELA or that subjects with smaller volume exposed to ELA are more prone to MDDSui. In all DG regions, ELA was associated with more GNs and a trend toward more glia in all subjects, while there was a trend to fewer NPCs with ELA history only in control subjects. In rodents, ELA is associated with more proliferation and neurogenesis shortly after exposure, while long-term effects are unknown (66). NPCs are in the thousands, while there are millions of GNs, and, together with changes in dendrite morphology (67,68) or spine density (69,70), they likely contribute to larger DG volume in resilient subjects. Larger DG volume could predispose to resilience or be the result of how the brains of control subjects react to ELA exposure.

Smaller volume in MDDSui-w/oELA than in Control-w/oELA in whole DG could be in part due to fewer GNs in MDDSui in anterior DG versus control subjects or, to a minor extent, to fewer NPCs in MDDSui-w/oELA versus Control-w/oELA in anterior DG. Nevertheless, smaller whole DG volume in MDDSui-w/oELA possibly implicates processes and dendrites, as shown in rodent depression models (71,72). We previously reported smaller DG in MDDSui control subjects (15,73), consistent with magnetic resonance imaging reports in unmedicated MDD (19). Specific roles of MDD and suicide in DG volume changes remain to be assessed.

Better GAS score correlating with more GNs and larger DG volume in anterior hippocampus supports our previous report of more lifetime depressive episodes and earlier MDD onset correlating with fewer anterior DG GNs (15). DG cell viability deficits in MDD could be a cause or consequence of a more severe form of MDD owing to increased glucocorticoid allostatic load (74). Neurogenesis has been implicated in cognitive performance and emotional responses in rodents (75,76). The clinical relevance of human adult neurogenesis has been debated (42,77,78). The recent controversy regarding the existence of adult neurogenesis in humans, with our laboratory showing thousands of NPCs and immature neurons in the DG of subjects up to 79 years of age (43) and another study finding no DG neurogenesis after adolescence (42), warrants further studies on this topic. Immunohistochemistry, immunofluorescence, and in situ hybridization results are affected by tissue preservation and processing methods and PMI, which alter proteins and RNAs and their reactivity to probes and antibodies (39,79). Moreover, some postmortem studies did not have information on neuropsychiatric diagnosis and did not perform toxicology screening (41,42,80,81). Neuropsychiatric diseases and use of drugs and medications directly affect amounts of neurogenesis in mice (82,83), rats (84), nonhuman primates (85), and human anterior DG (15,31,32,73). Assessing the whole hippocampus is necessary for unbiased cell quantification (86). Others have estimated that one third of human GNs are generated during adulthood, with an estimated average of 700 new GNs per hippocampus per day and an annual turnover of 1.75% of GNs in humans (80), supporting possible functional relevance of these new neurons.

ELA may confer vulnerability to MDD (1,87–89), suicide (2,3,5,90), bipolar disorder (91,92), anxiety (93), and schizophrenia (94), while stress resilience is protective against psychiatric illness (95,96). Lifelong changes in hypothalamic-pituitary-adrenal axis regulation are found in monkeys deprived of their mothers (97). Lasting effects on adult stem cell function vary depending on the age of ELA exposure from prenatal (98) to infancy (99,100) to adolescence (101); therefore, we selected subjects with ELA exposure before 15 years of age. Predictable stress during adolescence is associated with resilience later in life (102,103). Elevated glucocorticoid (24,74,104), excessive glutamate excitatory transmission (105,106), lower trophic factor levels (107–109), and alterations of neurotransmitter systems known to occur in MDD and suicide (110) alter hippocampal neurogenesis (111) and may explain our findings of fewer GNs in MDDSui and fewer NPCs in MDDSui-w/oELA in anterior DG. Lack of cellular or volume changes associated with ELA in MDDSui could indicate that hippocampal neuroplasticity changes associated with MDDSui are less driven by ELA and more the result of neurobiological differences related to the illness.

Successful adaptation after ELA (112) is associated with good intellectual functioning, effective emotional regulation, and an active coping style, possibly the result of or leading to brain circuit remodeling, including changes in DG cellular plasticity, encoding of emotion-related memories, and amygdala–prefrontal cortex connectivity. Larger DG volume and a trend toward fewer NPCs found in all regions only in ELA-exposed controls could be resilience effects shaping the behavioral response to stress, possibly through higher NPC proliferation earlier in life, close to stress exposure, leading to more GNs and possibly more neuropil later in life.

Postmortem human studies cannot establish cause and effect but can detect correlations of heuristic importance. Mouse models can determine cause and effect relationships of cellular changes associated with resilience and can help with developing new preventive treatments and testing developmental biological models of mental illness. We excluded all cases with positive toxicology for exposure to antidepressants and receiving antidepressant prescriptions during the 3 months prior to death because they cause neurogenesis increase in mice (82,83), rats (84), nonhuman primates (85), and perhaps patients with depression (32,73), an effect that likely is age dependent (113). Exercise effects on neurogenesis could not be quantified (114). The small sample size, unbalanced for sex, warrants larger studies including more women to confirm and expand on these results. ELA was limited to separation from parents or physical or sexual abuse and did not include neglect, emotional abuse, poverty, socioeconomic stressors, chronic illness, or imprisonment of a parent; therefore, our findings cannot be generalized to other forms of ELA.

Molecular mechanisms associated with changes in cell proliferation, maturation, and survival, process length, and synapse density in relationship to ELA exposure should be investigated in individuals who develop psychopathology and in resilient subjects. Specificity of findings for MDD versus suicide remains to be determined.

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