Residual Hippocampal Atrophy in Asphyxiated Term Neonates

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ABSTRACT

Background and Purpose. Previous studies have shown the hippocampus and basal ganglia to be highly sensitive to hypoxic-ischemic insult. The authors' aim was to evaluate the long-term effects of perinatal asphyxia (PA) on the hippocampus and caudate nucleus in a group of participants born at term and who met the criteria for hypoxic-ischemic encephalopathy (HIE). Additionally, the authors looked for damage in other brain regions using voxel-based morphometry (VBM). *Methods*. The sample consisted of 13 participants (8 boys and 5 girls) with a mean age at study of 16.23 years (± 2.89) with antecedents of perinatal asphyxia, diagnosed as moderate hypoxic-ischemic encephalopathy. A group of 13 healthy adolescents matched for age, sex, educational level, and social background were recruited as a comparison group. MR scans were acquired on a 1.5T Signa (General Electric, Milwaukee, WI) to evaluate hippocampal and caudate volumes and to perform VBM analysis. Finally, Rey's Auditory Verbal Learning Test was administered to evaluate verbal long-term memory. *Results*. HIE participants were found to have bilateral hippocampal atrophy (P = .015) and gray matter damage in temporal and frontal lobes. The caudate nucleus showed no atrophic changes in PA participants, and VBM analysis did not reveal other consistent brain abnormalities. Verbal long-term memory was slightly worse in HIE participants. *Conclusions*. These findings indicate that PA produces hippocampal and other nonspecific long-term damage, which cannot be compensated for by plasticity mechanisms. However, this damage does not preclude normal development and scholarship.

Key words: Hippocampus, hypoxic-ischemic encephalopathy, pediatric disorders, perinatal asphyxia, MRI, voxel-based morphometry.

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Perinatal asphyxia (PA) is a condition in which the newborn suffers an oxygen diminution (hypoxia) associated with high levels of carbon dioxide (CO_2) (hypercapnia) and a diminution of blood flow.[1] Moreover, it frequently produces lesions in cortical areas, basal ganglia, thalamus, and brainstem.[2,3] One region that is highly sensitive to hypoxic-ischemic damage is the hippocampus.[4] Neuroimage findings indicate that hippocampal damage in PA patients can persist throughout development,[5] and this has also been observed in volumetric[6] and

neurochemical[7] studies. Voxel-based morphometry (VBM), a method for evaluating the density of brain gray matter, has also shown hippocampal damage in adolescent patients with antecedents of PA.[8]

Although there is evidence of long-term sequelae in the hippocampus of patients with severe PA,[6,8] it would be interesting to know if this damage is also present in patients with a less severe PA episode, and where premature birth is not an associated complication, as the latter can also lead to MRI abnormalities and poorer cognitive outcome.[9]

To address this question, we investigated possible hippocampal atrophy in a selected sample of adolescents, born at term, with antecedents of perinatal asphyxia and moderate encephalopathy but without neurological sequelae, and with an IQ above the mean. We also analyzed bilateral caudate nucleus volume, as magnetic resonance spectroscopy studies have indicated possible basal ganglia involvement in PA.[7] To detect damage in other brain regions, we applied VBM. Finally, we administered Rey's Auditory Verbal Learning Test, as hippocampal damage sustained early in life can affect declarative memory,[6] and hippocampal integrity is related to declarative memory.[10]

Materials and Methods

Patients

The sample consisted of 13 patients (8 boys and 5 girls) with a mean age at study of 16.23 years (± 2.89) with antecedents of perinatal asphyxia. Patients were diagnosed as having moderate hypoxic-ischemic encephalopathy (HIE) using Sarnat and Sarnat criteria.[11] According to this classification, grade 2 corresponds to moderate HIE with the infant displaying lethargy, mild hypotonia, proximal weakness (ie, suck reflex weak or absent and moro reflex incomplete, with high threshold), and autonomic dysfunction (ie, miosis, bradicardia). Moreover, EEG abnormalities can be present, with focal or multifocal seizures. Patients' clinical data are shown in Mañeru et al.[7] With respect to this previous study, we excluded patients 9, 11, 13, and 14 because they did not fulfill criteria for moderate HIE, and patient 6 because a phobic reaction precluded the volumetric MRI study. Patients' development was normal, without neurological or psychiatric sequelae. All patients followed normal schooling (years of schooling: 10.92 ± 2.72).

A group of 13 healthy adolescents matched for age (mean age at study: 15.62 ± 2.99 years), sex, educational level (years of schooling: 9.92 ± 2.75), and social background were recruited as a comparison group. Signed, informed consent was obtained from all participants or their parents.

Magnetic Resonance Imaging

MR scans were acquired on a 1.5T Signa (General Electric, Milwaukee, WI). The protocol included axial T2Wdual FSE (4000TR/20-100TE/1nex/3mm slice thick) and coronal 3D (SPGR 300TR/min full TE/20 flipα/1nex/1mm slice thick. recon.), field of view 24 × 24, and matrix 256 × 256.

Morphometric Analysis

All image processing was performed by the same research fellow (JMS-G) who was blind to all clinical information.

Hippocampus

Following Bigler et al,[12] the posterior boundary of the hippocampus was identified as being (a) at the level of the superior colliculi and (b) where the oblong position of the hippocampus was visible at the level of the crura of the fornices. This is a conservative criterion, since a small portion of HCT was excluded from the segmentation. However, individual landmarks could be consistently identified. The anterior boundary of the hippocampus was identified at the level where the amygdala and hippocampus were distinguishable. It is important to note that the differentiation between the anterior hippocampus and the amygdala is difficult, since the posterior part of the amygdala is partly overlapping the anterior part of the hippocampal head.[13] As it was described in Pruessner et al,[14] the use of arbitrary landmarks to demarcate boundaries of the hippocampus results in a wide variety of outcomes, being difficult to compare between studies. To reduce such arbitrary landmarks, in our study we used the 3D protocol for manual segmentation of the hippocampus, which results in a more reliable segmentation. Overlapping between hippocampal head and posterior amygdala can be disclosed using simultaneous sagittal and coronal views, by using the alveus as a landmark for delimitation between the hippocampus and the amygdala. Figure 1 contrasts the hippocampi of a patient with antecedents of perinatal asphyxia with the hippocampi of the matched control.

Caudate Nucleus

The medial and lateral limits of the caudate nucleus were defined as the presence of the lateral ventricle and the anterior parts of the internal capsule, respectively. The superior boundary of the caudate nucleus was clearly identifiable by the presence of white matter. The inferior limit of the caudate nucleus was defined as the presence of the nucleus accumbens. This boundary was the most diffi-

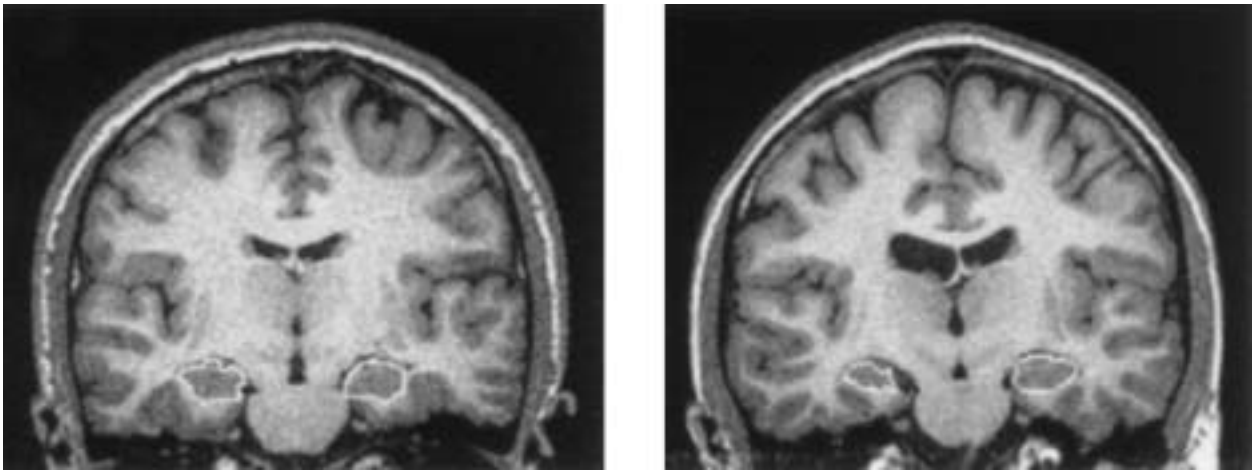

Fig 1. Comparative coronal MR images showing the hippocampi in patients with perinatal asphyxia (right side) and their matched control. In each image, left side corresponds to the right hemisphere, and right side corresponds to the left hemisphere.

cult to determine, since caudate and accumbens nuclei are very close ventromedially. To overcome these difficulties, we used the orthogonal sections tool of ANALYZE v.3.0 software (Mayo Foundation, Rochester, MN). This tool gives the coordinates in any spatial localization simultaneously in coronal, sagittal, and transversal views. In a coronal view, the caudate nucleus was delimited from the accumbens tracing a horizontal line from the more basal limit of the lateral ventricle. This can be considered a conservative criteria, since a small portion of the caudate region was excluded from the ROI to avoid including parts of the accumbens. In more posterior coronal slices, the caudate nucleus was easily distinguishable from other structures.

Measurements of the hippocampus and caudate nucleus were corrected for differences in total intracranial volume.[15] The intracranial volume was obtained after an automatic segmentation process, integrated in SPM99 (Wellcome Department of Cognitive Neurology, University College, London, UK).

Voxel-Based Morphometry Method

The VBM is a method for voxel-wise, between-group comparison of the local gray matter concentration.[16] The 3D MRI data sets were analyzed using the VBM method implemented in SPM99 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, UCL, London, UK),[17] running in Matlab 6.0 (MathWorks Inc., Sherborn, MA, USA). We followed a procedure described elsewhere.[18]

The T2-weighted and PD images were transformed into standard MNI (Montreal Neurological Institute) space using an automated spatial normalization. The normalized whole-brain images were automatically segmented into an image representing probability maps for gray matter (GM) and finally smoothed using an 8-mm full-width half-maximum isotropic Gaussian kernel.

Neuropsychological Assessment

We used a version of Rey's Auditory Verbal Learning Test (RAVLT), a memory test that has previously been found to be sensitive to hippocampal dysfunctions.[19] In the RAVLT, patients are instructed to recall a list of 15 common words in 5 trials. Immediate free recall was obtained by counting the total number of the 15 list words recalled on trials 1 to 5. Long-term recall was measured by the number of words recalled 30 minutes after the fifth trial. Recognition consisted of the identification of the 15 words among 30.

Statistical Analysis

We used MANOVA analysis of repeated measures to evaluate bilateral differences in hippocampal volumes and to evaluate performance differences between successive trials in RAVLT. The Student *t* test was used for independent samples to compare the group means with respect to both MR and neuropsychological variables. For all the analyses, we took the 2-tailed significance. Spearman correlation coefficients were computed to determine the relationship between volumetric measures and neuropsychological performance.

For VBM compare-populations analysis, we performed 2 1-sided t tests GM comparison (controls $>$ patients and patients $>$ controls). P values were derived

Table 1. Volumetric (mm^3) and Neuropsychological Measures in Control and HIE Patients

	Control Mean (SD)	HIE Mean (SD)	$t_{(1,24)}$	*P* Value
Volumetric measure				
Left hippocampus	2633.07 (316.09)	2342.09 (268.63)	2.52	.018
Right hippocampus	2744.79 (257.88)	2452.13 (343.77)	2.45	.022
Total hippocampus	5377.86 (563.90)	4794.23 (567.29)	2.63	.015
Left caudate nucleus	3841.39 (403.36)	3716.48 (516.77)	0.68	.499
Right caudate nucleus	3901.56 (393.91)	3858.88 (495.42)	0.24	.810
Total caudate nucleus	7742.95 (784.20)	7575.36 (991.57)	0.47	.637
RAVLT				
Words recalled				
Total IFR1-IFR5	57.00 (5.80)	52.46 (8.65)	1.57	.129
Trial 6 - LTR	13.31 (1.60)	11.62 (2.18)	2.25	.034
Recognition	15.00 (0.00)	14.23 (1.01)	2.74	.011

HIE = patients with hypoxic-ischemic encephalopathy; RAVLT = Rey's Auditory Verbal Learning Test; Total IFR1-IFR5: sum of hits on 5 trials.

for both differences in gray matter density on a voxel-by-voxel basis and the spatial extent of clusters of the affected voxel. Only those clusters that exceeded a size of 5 voxels were included in the analysis.

Results

Morphometric Results

MANOVA analysis of repeated measures shows a laterality effect of hippocampal volume ($F_{1,24} = 8.672$, $P = .007$), the right hippocampus being slightly larger than the left hippocampus. The factor group was significant, indicating that the hippocampal volume of HIE patients was smaller ($F_{1,24} = 6.921$, $P = .015$). Interaction analysis between the laterality and group factors did not reach significance ($F_{1,24} = 0.001$, $P = .982$). For post-hoc analysis of hippocampal volume, we performed the Student *t* test. Moderate HIE patients showed bilateral hippocampal atrophy. In Figure 2, we can observe the hippocampal values of each patient. On the other hand, cau
date nucleus volumetric analysis showed no differences between PA and control patients (Table 1).

Voxel-Based Morphometry Results

VBM analysis did not show gray matter differences in both comparisons (controls > patients and patients > controls) when the data were corrected for multiple comparisons. However, by using uncorrected P values < .001, the gray matter controls > patients comparison revealed that HIE patients had a reduction of gray matter in different brain regions, mainly of the temporal and frontal lobes (see Fig 3). Table 2 summarizes the results at uncorrected *P* values.

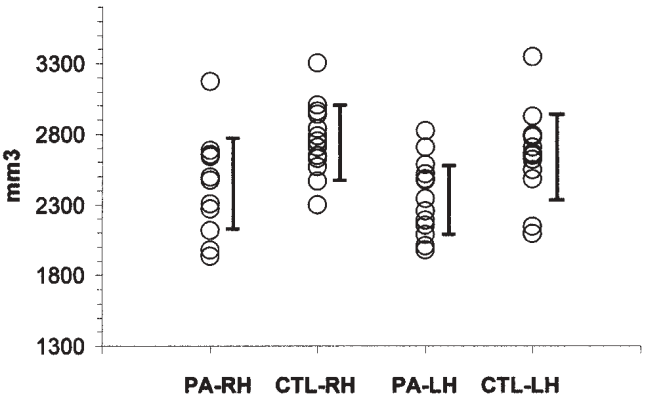

Fig 2. Right and left hippocampus volume for each patient with perinatal asphyxia (PA) antecedents and normal controls. Black vertical bars indicate mean volumes ± 1 standard deviation. CTL-LH = left hippocampal volumes of control patients; CTL-RH = right hippocampal volumes of control patients; PA-LH = left hippocampal volumes of PA patients; PA-RH = right hippocampal volume of PA patients.

Memory Outcome

Multivariate analysis of variance applied to immediate free recall in Rey's Auditory Verbal Learning Test showed significance for the repetition factor ($F_{4,24} = 135.28$, $P < .001$), indicating that all patients performed better on successive trials from trial 1 to trial 5. Analysis for the group factor did not reach significance ($F_{1,24} = 2.46$, $P = .129$), indicating that both groups performed similarly from trial 1 to trial 5. Moreover, analysis of group and repetition fac-

Table 2. Location of All Significant Points in the VBM Analysis at the Uncorrected *P* Value of $P < 0.001$

Region	X	Y	Z	$SPM_{\{t\}}$	*P* value
Precentral gyrus, left frontal lobe	–51	–6	31	4.83	< 0.001
Insular gyrus, left temporal lobe	–53	0	6	4.34	< 0.001
Left parahippocampal gyrus	15	–38	0	4.09	< 0.001
Cingulate gyrus, limbic lobe	12	–29	35	4.06	< 0.001
Inferior temporal gyrus, left temporal lobe	–55	–15	–21	3.94	< 0.001
Superior temporal gyrus, left temporal lobe	–49	–33	7	3.81	< 0.001
Superior temporal gyrus, right temporal lobe	53	–36	16	3.58	< 0.001
Superior frontal gyrus, right frontal lobe	26	56	23	3.47	< 0.001
Inferior frontal gyrus, left frontal lobe	–32	33	–8	3.38	< 0.001

X, Y, and Z columns represent Talairach coordinates (in mm).

tor interaction showed no significance ($F_{4,24} = 1.74$, $P = .146$). For post-hoc analysis of learning, we performed the Student *t* test, which showed that HIE patients performed worse on long-term recall (LTR) ($T_{1,24} = 2.25$, $P = .034$) and recognition ($T_{1,24} = 2.74$, $P = .011$) (see Table 1). For moderate HIE patients, Spearman correlation analysis between neuropsychological performance and left (LHV) and right (RHV) hippocampal volume did not reach significance; total immediate free recall with LHV: $r = –.24$, $P = .420$; total immediate free recall with RHV: $r = –.23$, $P = .442$; LTR with LHV: $r = –.26$, $P = .383$; LTR with RHV: $r = –.23$, $P = .448$; recognition with LHV: $r = –.45$, $P = .119$; recognition with RHV: $r = –.416$, $P = .158$.

Discussion

Volumetric analysis showed that HIE patients had left and right hippocampal atrophy, a finding consistent with previous results. Barkovich[5] found that hippocampal abnormality resulting from profound PA persists throughout development, but in that study hippocampal damage was neither quantified nor related to memory outcome.

Hippocampal damage was also quantified in 2 recent works.[6,8] However, the hippocampal reduction found in our study is more directly attributable to the hypoxic condition, as our PA cases were all born at term, whereas some patients in these 2 previous studies had prematurity as an associated complication. This is important, as prematurity has recently been shown to be associated with MRI abnormalities.[9] In a sample of 25 preterm patients compared with 39 group-matched term control children, the authors reported a hippocampal reduction of 16% on the left and 12% on the right, and a left and right basal ganglia reduction of 11.4% and 13.8%, respectively.

One possible explanation of hippocampal damage subsequent to perinatal asphyxia is related to glutamate release. A hypoxic-ischemic episode causes excessive presynaptic release of glutamate,[20] which has a high affinity for *N*-methyl D-aspartate (NMDA) receptors.[21] NMDA receptors are located abundantly in the hippocampus,[22] and persistent binding of glutamate has excitotoxic effects, causing cell death.[23]

VBM analysis did not show gray matter differences between PA and control participants at corrected *P* values. Failure to find hippocampal gray matter hypodensities and other subcortical abnormalities could be due to the fact that we had a PD/T2 slice thickness of 3 mm, which may impede the detection of subcortical abnormalities. However, temporal and frontal brain abnormalities found at uncorrected *P* values did not discard the possibility that moderate HIE causes long-term unspecific brain damage in these regions, as the software's correction for multiple comparisons (originally designed to analyze functional imaging data) is very strict when applied to the analysis of structural data.[24]

The results also indicate that the plasticity mechanism, for example, glial proliferation and neurogenesis in human hippocampal neurones,[25] is not able to compensate for hippocampal damage resulting from a hypoxic-ischemic episode.

With respect to memory assessment, we found that HIE patients performed slightly worse on long-term recall. This memory deficit is consistent with the view that damage to the hippocampus, an area involved in declarative memory,[26] can produce enduring memory impairment, especially of episodic memory,[6] which seems to be totally dependent on the hippocampus.[27,28] In terms of recognition, although PA patients scored lower, a recognition mean of 14.23 words is sufficiently high to be considered normal.

Finally, we did not find volumetric abnormalities in the HIE caudate nucleus. These data disagree with previous spectroscopic results, which indicate basal ganglia damage in PA patients.[7] This discrepancy can suggest that volumetric analysis is less sensitive than spectroscopy to detect subtle brain damage.

Conclusions

Findings from the present report indicate that PA can result in long-term brain damage, the hippocampus being a region that is highly sensitive to hypoxic insult. This hippocampal atrophy seems to be accompanied by a memory deficit and cannot be compensated for by plas-

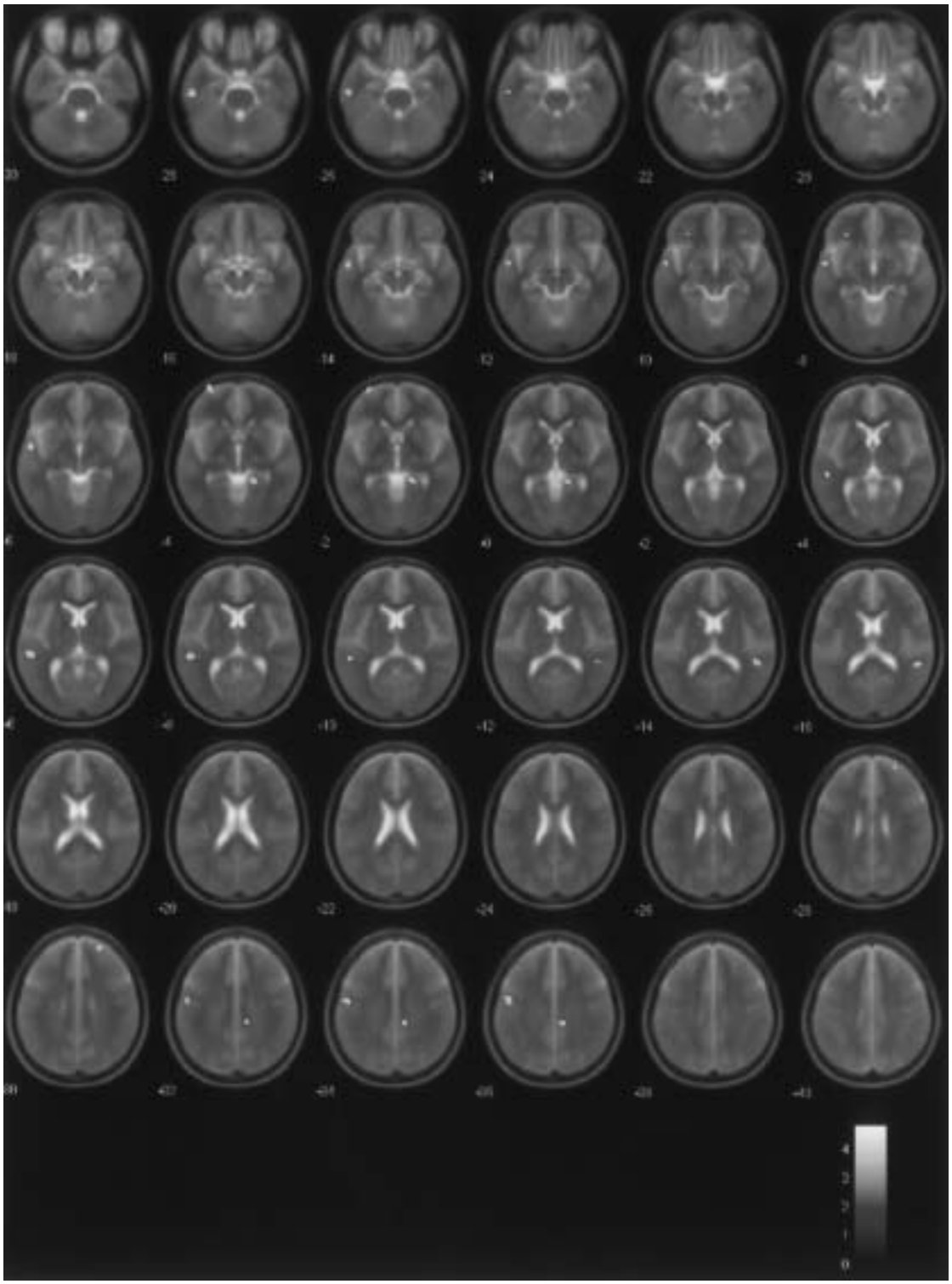

Fig 3. Statistical map of gray matter decreases in patients superimposed to an average image of all controls' volumetric T2 images ($P \leq .001$, $k = 5$, uncorrected). Different colors represent different t values (see color bar).

ticity mechanisms. However, in moderate HIE this damage does not preclude normal development and scholarship.

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