

Monkeys that Voluntarily and Chronically Drink Alcohol Damage their Brains: a Longitudinal MRI Study

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Neuroimaging has consistently documented reductions in the brain tissue of alcoholics. Inability to control comorbidity, environmental insult, and nutritional deficiency, however, confound the ability to assess whether ethanol itself is neurotoxic. Here we report monkey oral ethanol self-administration combined with MR imaging to characterize brain changes over 15 months in 18 well-nourished rhesus macaques. Significant brain volume shrinkage occurred in the cerebral cortices of monkeys drinking ≥ 3 g/kg ethanol/day (12 alcoholic drinks) at 6 months, and this persisted throughout the period of continuous access to ethanol. Correlation analyses revealed a cerebral cortical volumetric loss of $\sim 0.11\%$ of the intracranial vault for each daily drink (0.25 g/kg), and selective vulnerability of cortical and non-cortical brain regions. These results demonstrate for the first time a direct relation between oral ethanol intake and measures of decreased brain gray matter volume *in vivo* in primates. Notably, greater volume shrinkage occurred in monkeys with younger drinking onset that ultimately became heavier drinkers than monkeys with older drinking onset. The pattern of volumetric changes observed in nonhuman primates following 15 months of drinking suggests that cerebral cortical gray matter changes are the first macroscopic manifestation of chronic ethanol exposure in the brain.

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INTRODUCTION

The popular notion that ethanol itself is a neurotoxin responsible for brain damage seen with excessive alcohol consumption and alcohol dependence is little supported by direct evidence. Despite decades of rigorous studies reporting regional cortical volume shrinkage and neuronal dysmorphology *in vivo* (Cardenas *et al*, 2011; Fein *et al*, 2009; Pfefferbaum *et al*, 2012; Zahr *et al*, 2011) and in postmortem tissue (Harper, 1998; Zahr *et al*, 2011), the question whether excessive alcohol consumption *per se* is the cause of these brain structural abnormalities remains unestablished. Although longitudinal neuroimaging studies provide compelling evidence for partial to complete reversal of observed brain tissue shrinkage or ventricular expansion with sobriety and progressive dysmorphology with continued drinking in humans (Cardenas *et al*, 2005; Pfefferbaum *et al*, 1995; Rosenbloom *et al*, 2007), such studies are constrained due to ethical and practical

considerations when performed in human subjects and hence to their reliance on naturalistic observation. Thus, relevant factors, including premorbid assessment, environmental insult, nutritional deficiency, alcohol consumption patterns, comorbid psychopathology and other substance abuse, and the frequency of withdrawals cannot be controlled or often even adequately documented.

To address whether excessive, prolonged, and voluntary alcohol consumption *per se* can disturb brain structure, animal models are essential. Initially, we demonstrated reductions in the brain volume of ethanol-exposed rats relative to their baseline volumes and to controls (Pfefferbaum *et al*, 2006; Pfefferbaum *et al*, 2008). However, these rodent studies usually require forced exposure to ethanol to reveal the brain tissue reduction *in vivo* and therefore limit the translation to the human condition. Nonhuman primate models offer several advantages for the study of ethanol-induced brain damage. First, the brains of old-world monkeys, such as the rhesus macaque (*Macaca mulatta*) species studied here, are gyroencephalic and, like humans, have a large fraction of intracranial space occupied by white matter. Second, nonhuman primates are similar to humans in showing wide individual differences in average voluntary daily ethanol consumption, with a significant proportion categorized as chronic heavy drinkers and voluntarily drinking ethanol to physical dependence

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Table 1 Brain Region Definitions Relative to the Label Map Available through <http://nitrc.org/projects/inia19>

Regions	Labels
Cerebral cortex	{Frontal, sensory/motor, temporal, parietal, occipital, allocortex}
Frontal	196, 213, 214, 215, 216, 217, 220, 1196, 1213, 1214, 1215, 1216, 1217, 1220
Sensory/motor	111, 147, 1111, 1147
Temporal	37, 45, 54, 58, 1037, 1045, 1054, 1058
Parietal	7, 8, 9, 10, 1007, 1008, 1009, 1010
Occipital	1, 3, 4, 5, 6, 1001, 1003, 1004, 1005, 1006
Allocortex	63, 65, 70, 1063, 1065, 1070
Hippocampus	69, 450, 451, 453, 454, 457, 458, 459, 461, 462, 463, 467, 1069, 1450, 1451, 1453, 1454, 1457, 1458, 1459, 1461, 1462, 1463, 1467
White matter	2, 55, 97, 149, 150, 151, 193, 1002, 1055, 1097, 1149, 1150, 1151, 1193
Lateral ventricles	51, 73, 74, 201, 1051, 1073, 1074, 1201

(Cuzon-Carlson *et al*, 2011; Welsh *et al*, 2011). Third, macaques have relatively long life spans, allowing prolonged ethanol exposure (months to years) and enabling insight into mechanisms of long-term physiological adaptations, possibly modeling adverse biomedical outcomes of excessive alcohol consumption in humans (Cheng *et al*, 2010; Ivester *et al*, 2007; Shively *et al*, 2007).

Here we report longitudinal, structural neuroimaging data in well-nourished, physically healthy macaque monkeys *in vivo*, which underwent a standardized protocol (Grant *et al*, 2008; Vivian *et al*, 2001) for establishing heavy oral alcohol self-administration. The drinking protocol first induced the scheduled consumption of water and then ethanol (4% w/v), followed by concurrent access to water and 4% ethanol in daily 22 h sessions (termed ‘open-access’) for over 12 months (Grant *et al*, 2008; Vivian *et al*, 2001). Brain volumetric data were obtained at baseline and following 6 and 12 months of daily ethanol self-administration. To characterize regional brain volumetric changes, we constructed the INIA19 rhesus macaque brain atlas using T₁-weighted MRI data obtained from young to middle-aged adult male animals (Rohlfing *et al*, 2012) and have registered this atlas with the NeuroMaps nonhuman primate brain parcellation system (Bowden *et al*, 2012). We use this atlas in the present longitudinal experiment to test the hypothesis that brain tissue volume reductions in nonhuman primates are directly related to the amount of high-dose, chronic consumption of voluntarily consumed ethanol.

MATERIALS AND METHODS

Subjects

Eighteen of the 19 adult male rhesus monkeys described previously (Rohlfing *et al*, 2012) served as subjects for this study. One monkey was included in INIA19 but not included in this study because it was removed from the study due to (non ethanol-related) health concerns during the drinking phase of the experiment. The mean \pm SD of animal age at the beginning of the experiment was 7.2 ± 1.6 years. All procedures involving animals were conducted in accordance with the ‘Guidelines of the Committee on the Care and Use of Laboratory Animal Resources’ (National

Health Council, Department of Health, Education, and Welfare, ISBN 0-309-05377-3, revised 1996). Before their implementation, all procedures were reviewed by the Institutional Animal Care and Use Committee of the Oregon National Primate Research Center and deemed in compliance with all local, state, and national regulations pertaining to the humane use of animal subjects.

Ethanol Self-Administration

As described in detail in (Kroenke *et al*, 2013), all subjects were trained to orally self-administer ethanol using a standard protocol (Grant *et al*, 2008; Vivian *et al*, 2001). Drinking periods consisted of a 3-month training and induction phase, during which each monkey consumed an average of 1.0 g/kg ethanol per day, followed by two 6-month periods of 22 h daily access to 4% (w/v) ethanol, concurrently available with water.

MRI Procedures

The MRI protocols, described previously (Flory *et al*, 2010; Kroenke *et al*, 2013; Rohlfing *et al*, 2012), include a T₁-weighted sequence acquired at the month 0, month 9, and month 15 sessions. These correspond to the ethanol-naïve baseline, following 6 months of 22 h daily access, and following 12 months of 22 h daily access. Briefly, data were acquired using a 3T Siemens Magnetom trio MRI system. Acquisition settings for T₁-weighted images were TE = 4.38 ms, TR = 2500 ms, and TI = 1100 ms in an MP-RAGE pulse sequence (Mugler and Brookerman, 1990). Images were acquired with a resolution of 0.5 mm isotropic voxels. Between 4 and 6 such images were acquired per imaging session, and, following motion correction, these were averaged together for improved signal-to-noise ratio (SNR) before further processing. At each session, sedation for MRI procedures was initiated with 10 mg/kg ketamine, and anesthesia was maintained with 1.5% isoflurane under ventilation control.

Brain Volume Analysis

We have previously described the construction of the INIA19 rhesus macaque brain atlas and its interface with the

NeuroMaps parcellation system of Bowden *et al* (2012). The INIA19 atlas was constructed from the first, ethanol-naïve, time point for 18 monkeys used for this study (plus an additional monkey that did not complete the drinking experiment beyond the initial 3 months). Identical procedures to those described previously were followed to combine individual images for each animal and to perform intensity bias field corrections, brain extraction, and image segmentation.

The INIA19 T₁-weighted template image was nonrigidly registered to each individual image using multilevel B-spline-free form deformation as described in (Rohlfing *et al*, 2012; Rohlfing *et al*, 2010), and this operation facilitated the transfer of the NeuroMaps label map into the coordinate system of the individual image. For each time point, the tissue volumes within the structures listed in Table 1 were determined. These volumes were computed by the volume of the intersection of the label maps identified in Table 1 with the gray matter, white matter, or cerebrospinal fluid 'tissue' label maps resulting from the image segmentation operation. To control for individual variation in head size, brain region volumes are expressed as a percentage of the intracranial volume, which is the volume of non-zero voxels in the skull-stripped image. The INIA19 atlas is available from <http://nitrc.org/projects/inia19/>. All image analysis software developed by the authors at SRI and used for this study is available from <http://nitrc.org/projects/cmtk/>.

Statistics

A mixed-model, repeated-measures ANOVA was used to evaluate the effect of categorization as heavy or light drinker on brain tissue volume. As in our previous longitudinal analysis of magnetic resonance spectroscopy data acquired with these monkeys (Kroenke *et al*, 2013), the optimal model for the within-subject correlation in our longitudinal analysis was determined using the Bayesian information criterion. This resulted in the use of an unstructured covariance model. False discovery rate correction was used for multiple comparisons (Benjamini and Hochberg, 1995). Correlation analyses were additionally performed to assess whether significant associations were observed between brain region volume change, defined as the difference between baseline and 6 or 12 months 22h free access to alcohol, and alcohol consumption, quantified as mean daily intake. In each case, these analyses were used to assess whether significant change (increase or decrease in volume) was observed. In cases where significant effects were detected, the direction of the correlation (positive or negative) is stated. Effects were considered significant at the level $\alpha = 0.05$.

RESULTS

The monkeys were free of clinical pathologies unrelated to alcohol drinking over the period of the experiments. All monkeys ate three meals per day, maintained free-feeding body weight throughout the induction period, and increased in weight throughout the drinking phase of the experiment, as described in previous work (Grant *et al*, 2008; Vivian *et al*, 2001). The weights and the results of

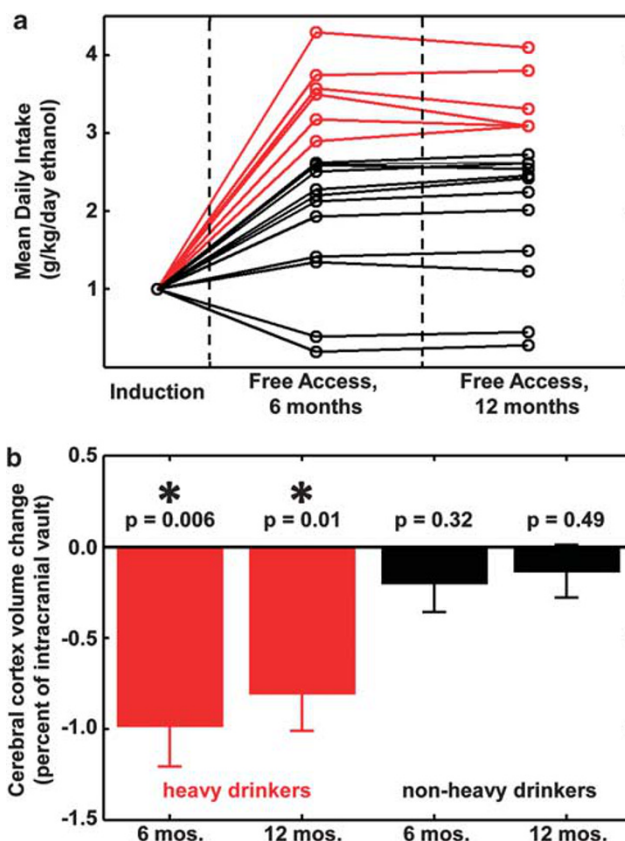


Figure 1 Drinking-dependent volume reductions of cerebral cortex in the rhesus macaque. (a) Mean daily ethanol intake for each of the 18 monkeys is shown over the course of the experiment. Throughout the 3-month ethanol drinking period, each monkey consumed 1.0 g/kg of ethanol per day. Subsequently, each monkey established a consistent mean daily intake, which was maintained throughout the year of voluntary drinking. Six of the 18 monkeys maintained a mean daily intake of greater than 3 g/kg ethanol throughout the voluntary drinking phase of the experiment and are categorized as heavy drinkers (red symbols/lines). (b) Significant reduction in the cerebral cortical volume, expressed as a percentage of intracranial volume (mean \pm SE), occurred in heavy drinkers (red bars) but in not non-heavy drinkers (black bars), following 6 and 12 months of 22h free access to alcohol.

clinical blood panels are given in Supplementary Tables S1 and S2, respectively.

Following the 3-month induction period, consistent with previous findings (Grant *et al*, 2008), each monkey self-sorted into a consistent within-individual average daily intake that varied considerably among monkeys (Figure 1a). By design, the mean daily ethanol intake was 1.0 g/kg during the induction period. Subsequently, over the first 6-month period of 22h open-access to ethanol and water, the individual mean daily intakes ranged from 0.2 to 4.3 g/kg, with a distribution mean \pm SD of 2.4 ± 1.1 g/kg. Of the 18 monkeys, 6 were categorized as heavy drinkers, based on our previous operational definition of consuming greater than 3.0 g/kg/day, approximately a 12 drink/day human equivalent (Grant *et al*, 2008; Vivian *et al*, 2001) (Figure 1a). The daily average intake for each monkey was consistently maintained over the second 6 months of open-access, and the distribution of mean daily intakes (2.4 ± 1.0 g/kg) remained constant.

In contrasting the effect of categorical heavy vs non-heavy drinking, average changes in the volume of cerebral cortical

gray matter, the hippocampus, cerebral white matter, and lateral ventricles were quantified in heavy and non-heavy drinkers by referencing the NeuroMaps nonhuman primate brain parcellation scheme (Rohlfing *et al*, 2012). To control for interindividual brain volume differences, volume measurements were expressed as a percentage of the intracranial volume. For cerebral cortical gray matter, significant reductions in average volume were observed following 6 and 12 months of 22 h open access to ethanol for heavy (6 months: $p=0.006$, 12 months: $p=0.01$) but not non-heavy drinkers (6 months: $p=0.32$, 12 months: $p=0.49$), as shown in Figure 1b. In contrast, the average volumes of white matter and lateral ventricles were unchanged at the 6 and 12 month measures for heavy and non-heavy drinkers. Hippocampal volume was not changed in heavy drinkers after 6 or 12 months of self-administration ($p=0.15$, and $p=0.18$, respectively). Likewise, average hippocampal volume was unchanged in non-heavy drinkers at 6 months ($p=0.17$); however, a significant increase following 12 months of ethanol self-administration ($p=0.02$) was observed.

To detect graded effects of ethanol exposure across the population of monkeys studied, analyses were performed to determine whether volume changes with drinking correlated with mean daily ethanol intake. As shown in Figure 2a, significant negative correlations were found between the mean daily ethanol intake and the change in cerebral cortical tissue, expressed as a percentage of intracranial volume, following 6 and 12 months of 22 h open access to ethanol. Although this analysis does not account for the possibility that brain tissue shrinkage is nonlinear across amounts of alcohol consumed and thus does not occur in low to moderate drinkers, the slopes of the Figure 2a lines correspond to 0.42 or 0.31% of intracranial volume loss per g/kg/day (or, ~ 0.11 or 0.08% of intracranial volume of cortical tissue per drink, 0.25 g/kg, per day), following 6 and 12 months 22 h free access, respectively. A further refined analysis was conducted to examine whether specific regions within the cerebral cortex were differentially affected by ethanol exposure. Applying the NeuroMaps annotation scheme (Rohlfing *et al*, 2012), the cortex was divided into frontal, somatosensory/motor, parietal, temporal, occipital, and allocortical regions (Figure 2b). Statistically significant negative correlations were observed between volume changes and mean daily ethanol intake within the parietal, temporal, occipital, and allocortical regions following 6 months of drinking, as indicated by red areas in Figure 2c.

After 12 months of continuous ethanol self-administration, the association between gray matter volume changes and daily ethanol intake was statistically weakened (Figure 2, Table 2). Although a significant negative correlation continued to be observed between cerebral cortical volume change and daily intake at 12 months ($r=-0.55$, $p=0.02$) the magnitude of the correlation coefficient was larger at 6 months ($r=-0.72$, $p=0.0008$; Table 2). Analyses of individual cortical regions indicated that correlations within regions besides the allocortex were not maintained using 12 months of average ethanol consumption (Figure 2d). Within the allocortex, a region that includes cingulate and fasciolar gyri (Figure 2b), as with the cerebral cortex overall, there remains a significant association between daily ethanol intakes and decreased

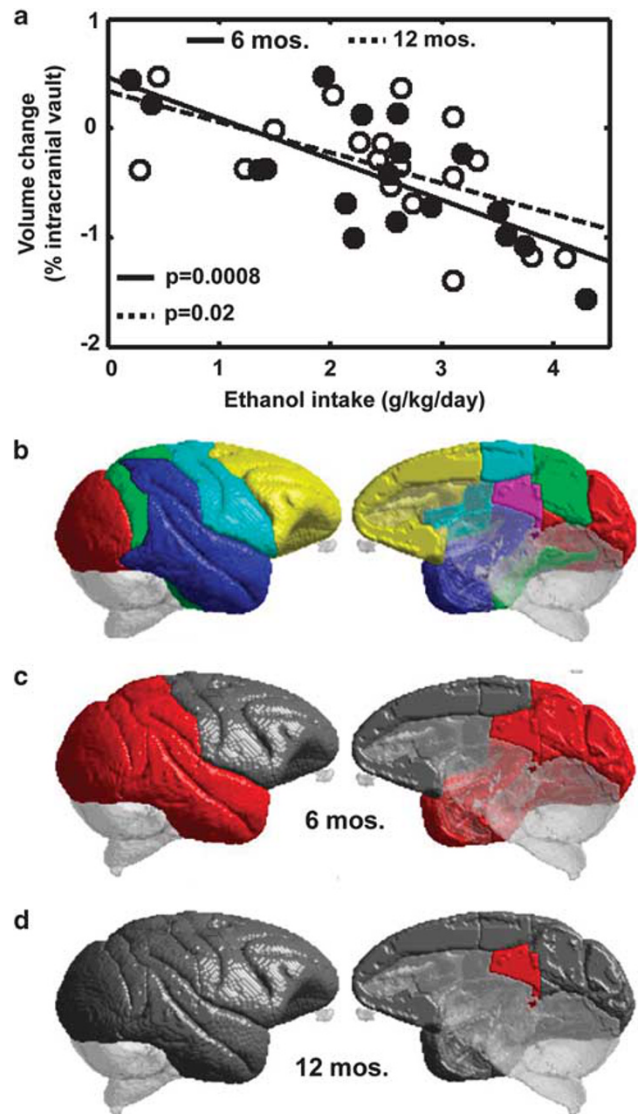


Figure 2 Correlations between cerebral cortical volume changes and daily ethanol intake. (a) Overall cerebral cortical volume change, expressed as percentage of the intracranial volume, as a function of mean daily intake for all monkeys following 6 months of drinking (solid symbols and lines) and following 12 months of drinking (open symbols/dashed lines). Cerebral cortical subregions were differentially susceptible to ethanol-related volume changes. (b) Following the NeuroMaps annotation scheme (Rohlfing *et al*, 2012), the cerebral cortex was parcellated into frontal (yellow), somatosensory/motor (light blue), parietal (green), temporal (dark blue), occipital (red), and allocortical (purple) regions. (c) Statistically significant negative correlations were observed between volume decline and higher mean daily ethanol intake within parietal, temporal, occipital, and allocortical regions following 6 months of drinking, as indicated by red areas. (d) Following 12 months of drinking a significant negative correlation is observed only between allocortical volume decline and higher mean daily ethanol intake.

volume at 6 and 12 month measures, but the association is lessened at 12 months (eg, the correlation coefficient is smaller in magnitude, and the p -value is larger, Figure 3a and Table 2).

Although the trends were generally in the expected direction, a number of statistically significant correlations were not observed between volumetric change and mean daily ethanol intake in the remaining brain areas.

Table 2 Pearson Correlation Coefficients and Associated *p*-values between Ethanol Daily Intake and Brain Volumetric Changes for all 18 Monkeys

Regions	6 Months		12 Months	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Cerebral cortex	-0.72	0.0008	-0.55	0.02
Frontal	-0.23	0.35	-0.22	0.39
Sensory/motor	0.42	0.08	-0.34	0.17
Temporal	-0.55	0.02	-0.26	0.30
Parietal	-0.52	0.03	-0.44	0.07
Occipital	-0.66	0.003	-0.36	0.14
Allocortex	-0.67	0.003	-0.46	0.05
Hippocampus	-0.66	0.003	-0.18	0.47
White matter	-0.39	0.11	0.02	0.95
Lateral ventricles	0.29	0.24	0.55	0.02

Bold values indicate *p* < 0.05.

Hippocampal volume change was significantly correlated with average daily ethanol intakes, with smaller volumes being associated with larger average daily intakes, following 6 months but not 12 months of drinking (Figure 3b, Table 2). Similarly, greater lateral ventricle volume expansion correlated with greater mean daily ethanol intake at 12 month but not at 6-month time point (Figure 3c, Table 2). Significant relationships between white matter volume changes and drinking were not observed (Table 2).

To examine the independent contributions of age and alcohol consumption on regional brain volume changes, we conducted a series of multiple-regression analyses. Age at the induction of ethanol drinking and mean daily intake at either 6 or 12 months were considered as simultaneous predictors of volume change in each of the brain areas. In no analysis did age itself contribute significantly to volume change in any brain region measured. By contrast, younger age at the beginning of induction predicted greater average ethanol intakes (g/kg/day) after 6 months (*r* = -0.57, *p* = 0.01) and 12 months (*r* = -0.59, *p* = 0.01) of open-access drinking. These results indicate that age-related brain volume changes do not contribute significantly to, or otherwise confound the volume reductions observed here.

DISCUSSION

These longitudinal MRI data provide unique direct evidence for chronic high-dose alcohol drinking to cause cortical volume loss in a nonhuman primate model of alcoholism. Specifically, monkeys that voluntarily drank an average of > 3.0 g/kg ethanol/day for 6 months sustained on average a loss of cortical gray matter of 1% of the intracranial volume. By contrast, cerebral cortices of monkeys that drank under an average of 3.0 g/kg ethanol/day did not differ in volume from baseline following 6 or 12 months of 22 h open access. Further support for alcohol as the cause of the observed brain changes for heavy drinkers was that the magnitude of the volume reductions was proportional to the mean daily ethanol intake. Ethanol's effect on the brain was not

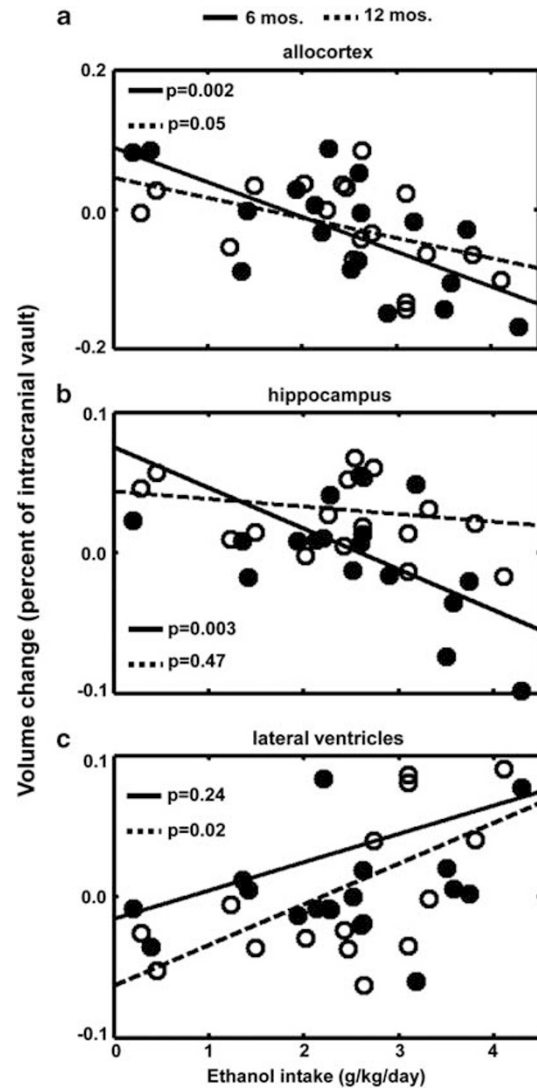


Figure 3 Brain volume change, expressed as percentage of the intracranial volume, is a function of mean daily intake for all monkeys following 6 months of drinking (solid symbols and lines) and following 12 months of drinking (open symbols/dashed lines). (a) Volume change of the allocortex is negatively correlated with mean daily intake following both 6 and 12 months of 22 h free access to 4% ethanol. (b) Volume change of the hippocampus is negatively correlated with mean daily intake following 6 months of drinking but the relationship between hippocampal volume change and drinking is not significant at 12 months. (c) Positive correlation between volumetric change in lateral ventricles and mean daily intake is statistically significant at 12 months but not 6 months.

generalized, but instead differentially affected specific brain regions. The posterior cingulate and fasciolar gyri of the allocortex showed the most consistent decrease in volume over time. In spite of likely changes at the level of neurotransmitter receptor mRNA expression (Acosta et al, 2011; Acosta et al, 2010; Hemby et al, 2006), the frontal cortex volume was observed to be independent of ethanol drinking. The hippocampus exhibited smaller volumes within the brains of heavier drinkers across the initial 6 months but not in the 12-month measures. Lastly, consistent with the human literature, the ventricular volumes increased following 12 months of 22 h open access in a manner proportional to the amount of ethanol consumed.

The value of conducting these experiments with nonhuman primate subjects is the explicit control over potentially influential factors, unachievable in human studies. Notable strengths include within-subject comparisons from the ethanol-naïve state through bouts of drinking, the precise measurement of ethanol intake, control over diet, and the age of first drinking to intoxication. This demonstration of a reduction in brain tissue volume as a function of ethanol consumed supports the position that at least a proportion of brain volume shrinkage observed in chronic alcoholics is related to alcohol consumption and not solely a premorbid condition or a result of other factors, such as head injury, dietary deficiency, illicit drug use, smoking, or psychiatric comorbidities.

Brain volume changes were largely restricted to gray matter structures in these monkeys. Although macroscopic differences in white matter structure have been reported between human alcoholics and controls (Pfefferbaum *et al*, 1992), such volume shrinkage was not evident herein. Of potential relevance to this point, however, Fein *et al* (2002) reported gray matter volume reductions in the absence of white matter volume reductions in treatment-naïve alcohol-dependent individuals. Fein and co-workers attributed the difference between their study and those of other treatment-seeking alcoholics, in which the white matter volume shrinkage was observed, to the possibility that brain volume changes are less severe in the general population of alcoholics than within hospitalized alcoholics. It is therefore noteworthy that the animals used in this study are likely more reflective of the general population than a selected distribution of individuals that have actively sought treatment for alcohol dependence.

Compared with the widespread gray and white matter volumetric reductions and compensatory increase in CSF spaces reported in human alcoholics (following decades of excessive drinking) (Pfefferbaum *et al*, 1998), the pattern of volumetric changes observed in nonhuman primates following approximately 1 year of drinking suggests that the cerebral cortical gray matter changes are the first macroscopic manifestation of chronic ethanol exposure in the brain. In a recent ^1H magnetic resonance spectroscopy (MRS) study of the same group of animals, it was found that the brain ethanol methyl ^1H MRS intensity per unit ethanol increased following chronic ethanol exposure (Kroenke *et al*, 2013). This effect on MRS intensity likely results from an increase in the ethanol methyl ^1H T_2 value, which is sensitive to biomolecular interactions between ethanol and macromolecular constituents of the brain tissue. Hence, the observed ethanol MRS effects indicate neuroadaptive changes related to ethanol molecular interactions take place following the drinking procedures described herein. Notably, parallel to the brain volume results described here, the ethanol methyl ^1H MRS intensity localized to the gray matter was responsive to chronic ethanol exposure at an earlier point in the experiment than were MRS changes within the white matter. Diffusion tensor imaging studies performed in humans have identified microstructural differences associated with ethanol exposure that take place in the absence of macroscopic manifestations (Pfefferbaum and Sullivan, 2002). Therefore, it is possible that undetected microstructural changes occurred within the white matter of the monkeys studied here earlier in their drinking history,

and such changes could manifest as white matter volume changes after longer periods of drinking.

The volumetric measures of the hippocampus reveal a dynamic process, with lower hippocampal volumes being observed in monkeys with higher mean daily ethanol consumption following 6 months of open-access drinking but no relationship between hippocampal volume change and drinking after 12 months. This pattern is in contrast with the cerebral cortex, which exhibited significant negative correlations with drinking at 6 and 12 months. Given the ability of hippocampal neurons to undergo morphological changes in response to stress (McEwen, 1999), and the accentuated role of adult neurogenesis in its maintenance (Nixon, 2006), it is possible that biological processes specific to the hippocampus underlie neuroadaptations occurring between 6 and 12 months of drinking. Alternatively, increased hippocampal volume following 6 months of drinking may reflect an inflammatory process associated with the continued exposure to alcohol (Crews and Nixon, 2009). Further investigations of neuroendocrine and neurocellular changes in the hippocampus matched to the imaging data will be necessary to explicate the complex changes observed here.

Age is an additional factor that leads to brain tissue volume decline in humans (Pfefferbaum *et al*, 1994; Pfefferbaum *et al*, 2013; Raz *et al*, 2010; Raz *et al*, 2005). The animals studied here ranged in age from 5.5 to 9.6 years at the beginning of the induction period, with a mean \pm SD of 7.2 ± 1.6 years. This corresponds to the rhesus monkey life span of young adult to the beginning of middle age. We considered it possible for two potential factors to mediate the correlation between age and volumetric changes reported here. The first is an effect of age on the brain volume as a consequence of the brains of older adults being more vulnerable to ethanol's effects (eg, Pfefferbaum *et al*, 1992). The second factor is that human epidemiological studies (Dawson *et al*, 2008; Grant *et al*, 2006) report that young adults, commensurate with the younger monkeys studied herein, are at greater risk than older adults to drink at hazardous levels. Multiple-regression analyses conducted here indicated that the latter of these two factors was observed. Younger age at drinking induction was associated with greater voluntary alcohol consumption and more dramatic brain volume loss compared with monkeys with relatively older ages at induction, which drank less alcohol and exhibited more modest tissue shrinkage.

Given its close similarity to many aspects of human drinking and brain morphology, this monkey model offers a translational platform to capture the initial dynamic stages of neuroadaptation to chronic ethanol consumption. We were able to characterize a time frame (6 months) and a threshold dose of heavy alcohol consumption (> 3.0 g/kg/day) that results in significant decrease in the cerebral cortical volume. The data also revealed a regional pattern of insult to selective cortical regions measured, rather than nonspecific effects, which is a finding that is strengthened by the within-subject longitudinal design. Further modeled was the high volume of youthful voluntary drinking, which in these monkeys resulted in substantial brain tissue volume shrinkage. This primate model will now enable direct testing of whether, when, and to what extent this volume loss is recoverable with the cessation of chronic alcohol consumption.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)