Plasticity of Hippocampal Subfield Volume
Cornu Ammonis 2+3 Over the Course of Withdrawal in Patients With Alcohol Dependence

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**IMPORTANCE** Research focusing on plasticity has shown adult neurogenesis in hippocampal subfields. Chronic alcoholism is associated with decreased plasticity and reduced whole hippocampal volume that could contribute to neuropsychiatric characteristics and outcome of the disease.

**OBJECTIVE** To investigate the effect of alcohol abstinence on neuronal plasticity measured as longitudinal volume change in distinct hippocampal subfields.

**DESIGN, SETTING, AND PARTICIPANTS** We acquired high-resolution structural images of 42 patients addicted to alcohol and 32 healthy control participants. Patients and control participants were both scanned twice, once after withdrawal and 2 weeks later.

**MAIN OUTCOMES AND MEASURES** Volumes of hippocampal subfields cornu ammonis (CA) 2+3, CA4+dentate gyrus, and subiculum were determined with a user-independent segmentation method.

**RESULTS** We found plasticity effects in bilateral CA2+3 in patients addicted to alcohol. Compared with healthy control participants, patients had lower CA2+3 volume at pretest ($t_{31} = −0.73, P = .47$) and showed a significant normalization of gray matter volume 2 weeks later. Pretest CA2+3 ($t_{31} = −3.93, P < .001$) volume was negatively associated with years of regular alcohol consumption ($r_{42} = −0.32, P < .05$) and more severe alcohol-withdrawal symptoms ($r_{38} = −0.35, P < .05$). Patients with stronger withdrawal symptoms displayed the largest volume increase of CA2+3 ($r_{38} = 0.55, P < .001$).

**CONCLUSIONS AND RELEVANCE** The observed normalization of the bilateral hippocampal CA2+3 volume deficit matches animal data, showing a strong increase of hippocampal neurogenesis after cessation of alcohol consumption, and fits the reported increase of patients’ cognitive function within a few months of alcohol abstinence. The role of CA3 in pattern separation and completion is also critical for formation of hallucinations, which constitute a severe symptom of the withdrawal syndrome. The study adds further biological arguments from structural brain research to abstain from alcohol.
The human brain has the striking capacity to adapt to changing environments by altering its structure; this process has been termed plasticity. The hippocampus is one of the most extensively studied brain regions of synaptic plasticity and experience-modulated behavior. Research has started to use neuroimaging techniques, such as magnetic resonance imaging (MRI), to investigate plasticity processes in the human brain in vivo. Adult plasticity provides a novel way to consider neurodegeneration and recovery in psychiatric disorders. In this study, we explored the capacity of the hippocampus to recover during the period of alcohol withdrawal in patients addicted to alcohol. Alcohol-use disorder is known to result in cognitive deficits that correspond to various functional and structural neurological pathologies. The impairments in functions, such as short-term and declarative memory, and spatial learning and memory suggest hippocampal pathology. Data concerning hippocampus integrity in individuals with alcohol dependence vary. Most of the previous volumetric studies seemed to suggest hippocampal cell volume loss in individuals with alcohol dependence. However, some studies failed to show differences between the hippocampus volume in patients with alcohol dependence and healthy control participants. The findings of hippocampus volume decreases are paralleled by descriptions of hippocampal cell loss and dentate gyrus (DG) neurodegeneration in animal models. Volumetric recovery during sobriety has been observed in several brain regions. There is evidence in humans suggesting that the entire hippocampal volume may increase over a 1-month period of abstinence from alcohol. In a rat model showing alcohol dependence, namely a chronic binge exposure to ethanol for 4 days, results indicated that adult neurogenesis in the hippocampus is inhibited during dependence and then reactivated considerably after weeks of abstinence. More precisely, rats showed a 4-fold increase in cell proliferation at day 7 of alcohol abstinence. Increases in cell proliferation correlated positively with withdrawal severity. From the animal literature it is known that the hippocampus is not a homogeneous structure but consists of several histologically distinct subfields including the cornu ammonis (CA) regions 1-4 DG and subiculum. The existence of different pathways within the hippocampus and different histological characteristics suggests that the subfields might house distinct aspects of cognitive processes. Given the evidence for subfield specialization, we set out to investigate the early effects of abstinence in patients addicted to alcohol on hippocampal subfield volume and its association with withdrawal severity and years of regular alcohol consumption.

Methods
Participants
A total of 42 patients dependent on alcohol were recruited during inpatient alcohol-withdrawal treatment at the Charité University Medicine Berlin, Berlin, Germany, as part of the National Genome Research Network (NGFN-Plus). In addition, 32 healthy control participants were recruited by newspaper advertisements. Patients’ inclusion criteria were DSM-IV diagnosis of alcohol dependence via Structured Clinical Interview for DSM-III-R rating and completion of medically supervised detoxification. Healthy control participants had no history of psychiatric disorders according to a Structured Clinical Interview for DSM-III-R. The Alcohol Use Disorders Identification Test indicated that healthy control participants did not have alcohol abuse or dependence. Demographic and drinking data of the participants are provided in the Table. In addition, exclusion criteria for all participants were abnormalities in the MRI, general medical or neurological disorders, or any clinically relevant abnormalities. All procedures of this study were approved by the local ethics committee at the Charité University Medicine, Berlin. Informed written consent was obtained from all participants.

Study Design
Participants were scanned twice, after withdrawal and 2 weeks later. The first measurement was performed after a mean (SD) of 9.4 (4.2) days and the second measurement after a mean (SD) of 23.2 (4.9) days of abstinence. To assess the years of regular alcohol consumption, we used the Lifetime Drinking History test. To quantify the severity of alcohol withdrawal syndrome, we used the Clinical Institute Withdrawal Assessment.

MRI Acquisition
Structural images were collected on a 3-T Verio MRI scanner system (Siemens Medical Systems) using a 12-channel radio frequency head coil. High-resolution anatomical images were acquired using a 3-dimensional T1-weighted magnetization-prepared gradient-echo sequence (repetition time = 2.3 milliseconds; echo time = 3.03 milliseconds; flip angle = 9°; 256 × 256 × 192 matrix, 1 × 1 × 1 mm voxel size). There was no major scanner upgrade during the study, and patients and control participants were scanned consecutively. All scans were visually inspected to exclude images with brain pathology.

Hippocampus Subfield Segmentation
The FreeSurfer software version 5.2.0 (http://www.surfer.nmr.mgh.harvard.edu/) was used to obtain volumes of the hippocampal subfields. The technical details have been previously described in detail. Briefly, the processing includes removal of nonbrain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures by combining information on image intensity, probabilistic atlas location, and the local spatial relationships between structures to automatically assign a neuroanatomical label to each voxel in the MRI volume. The hippocampal subfield segmentation is based on a Bayesian modeling approach and manual delineations of each hippocampal subfield. A cuboid region of interest around the hippocampal formation (94 × 66 × 144 voxels) is automatically assigned to each image using an affine mutual information-based registration technique by first aligning the wholebrain template and then the region of interest template only (see article by van Leemput et al for further technical details). The hippocampal subfield volumes obtained with this method have been compared with manual hippocampal sub-
field tracings and were shown to be most reliable for the larger subfields CA2+3, CA4+DG, and subiculum.29 Hence, we chose to include these subfields into the statistical analyses of the present study. The MRI postprocessing procedures were fully automated without manual editing. All segmented volumes were visually inspected.

Results

When investigating plasticity processes in the biggest and most reliably estimated subfields of the hippocampus (CA2+3, CA4+DG, and subiculum), we found a significant main effect of time ($F_{1,72} = 12.15, P < .01$) and a significant interaction of the factors group (patients vs healthy control participants) and time (after withdrawal vs 2 weeks later) in the subfield CA2+3 ($F_{1,72} = 8.04, P < .01$). This indicates that patients dependent on alcohol have smaller CA2+3 volumes at the start of the abstinence phase and normalize toward the volume of the healthy control participants over 2 weeks after withdrawal (Figure 1). Because the sex distribution was significantly different between groups, we entered sex as a covariate of no interest and confirmed the significance of the interaction effect ($F_{1,71} = 7.16, P < .01$). The statistical values survived Bonferroni correction for multiple testing of 3 subfields. In post hoc comparisons of CA2+3 volume, the difference between patients addicted to alcohol and healthy control participants was significant at pretest ($t_{39} = -3.93, P < .001$), and the change within the patients addicted to alcohol reached significance ($t_{39} = 2.16, P < .05$). The group difference was not significant at the end of the withdrawal period, indicating normalization of the hippocampal subfield volume ($t_{39} = -0.73, P = .47$). To test for lateralization effects, we computed the group by time interaction for right and left CA2+3 separately. The interaction effect was significant in both hemispheres (left: $F_{1,77} = 6.71, P < .05$; right: $F_{1,77} = 5.02, P < .05$).

To examine the association between CA2+3 volume and drinking behavior, we computed a correlation between pretest CA2+3 volume and years of regular alcohol consumption as assessed by the Lifetime Drinking History test. We found a significant negative correlation between years of regular drinking and CA2+3 volume ($r_{39} = -0.32, P < .05$) (Figure 2). Interestingly, we found an association between CA2+3 volume and the maximal score in the severity of alcohol-withdrawal syndrome assessment using the Clinical Institute Withdrawal Assessment. The smaller the CA2+3 volume at the outset of the abstinence phase, the higher the maximal severity of withdrawal ($r_{39} = -0.35, P < .05$). Moreover, the higher the maximal severity of the withdrawal symptoms, the greater the volume increase was in CA2+3 across the 2-week abstinence period ($r_{38} = 0.55, P < .001$).

Discussion

In this study, we demonstrated structural plasticity effects in the CA2+3 subfield of the hippocampus over the course of 2 weeks of abstinence in patients dependent on alcohol. Compared with healthy control participants, patients dependent on alcohol had lower CA2+3 volume at the start of the abstinence period and showed a significant normalization of gray matter volume over the subsequent 2 weeks, resulting in the elimination of significant group difference. Interestingly, CA2+3 volume was negatively associated with years of regular alcohol consumption in the patient population. Furthermore, those patients with smaller CA2+3 volume had more severe alcohol-withdrawal symptoms. In turn, patients with stronger withdrawal symptoms displayed the greatest plasticity effects of CA2+3 volume over the period of abstinence.

Our finding of a volume difference in CA2+3 between patients dependent on alcohol and healthy control participants...
is in line with previous studies reporting whole-hippocampus group differences in the same direction.9-14 The reverse correlation between years of regular alcohol consumption and CA2+3 volume, indicating that higher alcohol intake is associated with more pronounced volume deficit, is in line with previous reports of correlations between duration of alcoholism and hippocampal size.12

As suggested by Gazdzinski and colleagues,20 with respect to their finding on the whole hippocampus, the volume increase in CA2+3 over the course of abstinence could be based on rearborization of hippocampal dendrites,31-34 generalized changes in the neuropil, or glial regeneration.35 In studies on rats where a model of alcohol dependence was used that involved chronic binge exposure for 4 days, neurogenesis was shown to be inhibited during the dependence phase.21 On the other hand, during abstinence, a 4-fold increase in novel hippocampal neuron formation was observed, albeit in DG and the subgranular zone; to our knowledge, CA2+3 has not been investigated thus far.21,36 Similar to the present findings in humans, previous investigators reported higher neurogenesis was found in rats with stronger withdrawal symptoms. The so-called alcohol-withdrawal syndrome comprises a large range of behavioral and physiological signs that occur in alcohol-dependent individuals after the cessation of drinking.37 The most visible signs, such as tremor and seizures, have been subsumed under the term central nervous system-hyperexcitability, resulting from neural adaptations to chronic alcohol abuse.38 Previous animal studies have shown that seizures can result in proliferation of neural stem-progenitor cells and aberrant neurogenesis.39,40 However, Nixon and Crews21 found that reducing withdrawal severity by administration of diazepam in rats did not alter the observed cell proliferation; rats with low withdrawal severity did not differ from rats with high severity. On the other hand, our data on humans suggest that the higher the withdrawal severity, the higher the plasticity effect observed in CA2+3.

However, it is possible that it is not the withdrawal severity but factors such as the associated changes in psychological stress or diet that contribute to increased cell proliferation after the cessation of drinking. Stress and glucocorticoids have been shown to reduce neurogenesis41,42 and affect hippocampal volume in alcoholism.43 Moreover, it has been shown that binge ethanol ingestion elevates glucocorticoids during intoxication while the levels return to control values after withdrawal.44 Therefore, it could be fruitful to study stress regulation of the hypothalamic-pituitary-adrenal axis as a potential factor related to structural plasticity in patients recovering from alcoholism.

The functional implications of compensatory plasticity and potential neurogenesis during abstinence remain speculative until the function of adult neurogenesis is better understood.

Figure 1. Illustration of the Segmentation and Changes in Bilateral Cornu Ammonis 2+3 Volume

A. Illustration of a cornu ammonis 2+3 segmentation for a patient addicted to alcohol overlayed onto a magnetization-prepared gradient-echo image. B. Bilateral cornu ammonis 2+3 volume change in patients addicted to alcohol and healthy control participants in number of voxels (0.5 mm isotropic). aSignificant post hoc comparisons.

Figure 2. Scatterplot of Correlation

The negative correlation between regular alcohol consumption in years and cornu ammonis 2+3 volume at pretest ($r_{22} = -0.32, P < .05$).
However, one may speculate that neurogenesis in the hippocampus can contribute to learning and memory, because the inhibition of neurogenesis has been shown to disrupt associative learning. In line with this, previous studies have shown that abstinent individuals previously addicted to alcohol can indeed display improved cognitive function within a few months of abstaining from alcohol; however, the acute phase of withdrawal seems to be associated with more pronounced memory problems.

One of the classically ascribed roles of the hippocampus in memory is in binding stimulus elements into unitary representations. In rodent studies and neurocomputational models, the DG and its projections to CA3 and then to CA1 have been identified as neural substrates of pattern separation and are referred to as the trisynaptic circuit. Pattern separation is a critical feature of episodic memory and encompasses the ability to discriminate similar experiences. Recognition memory typically requires that rapidly stored memories must be unique in the face of interfering episodes. An example where we experience interference is when we memorize where we parked our car today when still having in mind where we parked our car yesterday. The assumed underlying mechanism is that cortical inputs are orthogonalized as they enter the hippocampus, activating a sparse network of selective units that can be used for pattern completion later on. There is consensus indicating that the DG responds to relatively small changes in input, potentially driving pattern separation signals in the CA3. Although nonlinear just like the DG, dynamics in CA3 can additionally exhibit pattern completion if the change in input pattern is small. On the other hand, CA2 has been ignored and is notably absent from the literature and many hippocampal circuit models. It has often been regarded as a distal part of the CA3 field. Other authors have suggested that CA2 contributes to signal propagation from CA3 to CA1 and could therefore fulfill a gating function.

Future studies on plasticity effects during the withdrawal period should associate changes in memory and learning with the observed effects in hippocampal subfields to get a grasp at the functional and day-to-day relevance of the observed plasticity effects in CA2+3.

Conclusions

To summarize, the present results suggest that the deficits in CA2+3 volume in patients with alcohol dependence compared with healthy control participants normalize over an abstinence period of 2 weeks. The negative association between CA2+3 volume and years of regular alcohol consumption suggests a dose dependency of the observed volumetric effect. The fact that lower CA2+3 pretest volume was associated with stronger withdrawal symptoms and that higher withdrawal severity was associated with stronger plasticity effects may motivate further studies investigating the causal link between specific withdrawal symptoms and neural plasticity effects during alcohol abstinence.
Plasticity of Hippocampal Subfield Volume CA2+3 in Alcoholic Patients

Original Investigation

Research

induced by prolonged ethanol consumption in rats. Science. 1980;209(4457):711-713.


