

# Hippocampus Volume Loss Due to Chronic Heavy Drinking

Thomas P. Beresford, David B. Arciniegas, Julie Alfers, Lori Clapp, Brandon Martin,  
Yiping Du Dengfeng Liu, Dinggang Shen, and Christos Davatzikos

**Objective:** No clear consensus exists regarding the effect of sustained, heavy drinking on hippocampal volume. Our prior work hypothesized significantly lowered total hippocampus volumes in heavy chronically drinking alcohol-dependent (AD) subjects compared with light-drinking nondependent control subjects matched for age and gender.

**Method:** Using a series of applicable exclusion criteria culled from previous published studies, we measured hippocampal volumes from MRI scan data acquired on a 3T scanner and subjected those data to automated volume analysis blind to the drinking history.

**Results:** Comparison with AD test ( $n = 8$ ) and non-AD control ( $n = 8$ ) subjects found significant lessening in total ( $p = 0.020$ ) and left ( $p = 0.010$ ) hippocampal volumes with a near-significant difference on the right ( $p = 0.051$ ). Linear regression demonstrated that neither total brain volume nor intracranial volume affected the hippocampus measures.

**Conclusions:** These data support the view that heavy drinking exerts a unique and selectively injurious effect on the hippocampus. Further study in larger samples must verify this in a search for possible mechanisms of injury.

**Key Words:** Hippocampus, Alcohol Drinking, Volume Loss, MRI Scan.

THE EFFECT OF sustained, heavy drinking on hippocampal volume is a subject of continued controversy. Although some reports in the literature point to a volumetric reduction in alcohol-dependent (AD) patients (Bleich et al., 2003a; Sullivan et al., 1995), others are less conclusive (Agartz et al., 1999). Some have argued that lack of precise sample definition has generated this confusion, citing inclusion of subjects with histories of withdrawal seizures (Sullivan et al., 1996), for example. Others have disputed such claims (Bleich et al., 2003b).

With development project funding, we began a prospective study of the hippocampal volume in chronically drinking AD subjects. To assess volume comparisons at baseline, we analyzed new data from heavy-drinking AD test and light-drinking control cases. Early data from

convenience samples of heavy drinking and control subjects (Beresford et al., 1999) led us to hypothesize that the mean total hippocampus volume (THV) in the AD subjects would be significantly smaller than the mean THV in the non-AD control subjects. Our specific aim was to replicate the findings of the prior study but in prospectively gathered sample groups utilizing more stringent exclusion and inclusion criteria.

## METHOD

This project received prior approvals from our university institutional review board (IRB) as well as from the Research and Development Committee of our Department of Veterans Affairs (DVA) facility where the study was conducted. All subjects were voluntary and signed preapproved consent-for-study documents, consistent with IRB policy.

### Inclusion/Exclusion

Both test (AD) and control subjects were adult male veterans who were eligible for care in the DVA system and were recruited in response to posted flyers advertising the study. Our research design matched AD test and non-AD control subjects for age, gender, and ethnicity. Alcohol-dependent heavy drinkers qualified for study if they met all of the following criteria:

- (1) *Chronic heavy drinking:* drank 5 or more standard drinks daily for at least 3 days weekly, and 3 weeks monthly for at least 9 months of the previous year, established through Time Line Follow Back interview (Sobell et al., 1979);
- (2) *Recent heavy drinking:* consumed 5 or more standard drinks daily on at least 3 days weekly for the past 30 days, established through TLFB;
- (3) *AD diagnosis:* fulfilled DSM-IV criteria for AD as established through the Structured Clinical Interview for DSM-IV Axis I

From the Mental Health Service, Department of Veterans Affairs Medical Center, University of Colorado School of Medicine, Denver, Colorado (TPB); the Department of Psychiatry, University of Colorado School of Medicine, Denver, Colorado (TPB, DBA, JA, LC, BM, YD); the Department of Neurology, University of Colorado School of Medicine, Denver, Colorado (DBA); the Department of Radiology, University of Colorado School of Medicine, Denver, Colorado (YD); and the Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania (DL, DS, CD).

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Reprint requests: Thomas P. Beresford, MD, Denver VA Medical Center (116), 1055 Clermont Street, Denver, CO 80220; Fax: 303-315-5641; E-mail: thomas.beresford@uchsc.edu

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Disorders (Kessler et al., 2004; Peters et al., 1998; Sbrana et al., 2003; Ventura et al., 1998) (SCID).

Similarly, non-AD light drinking comparison subjects presented TLFB histories of

- (1) <2 standard drinks daily for no more than 3 days weekly, 4 weeks monthly for 9 months or less during the previous year and
- (2) Drinking less than 2 standard drinks daily no more than 3 days weekly for the previous 30 days.
- (3) None fulfilled DSM-IV criteria, either present or lifetime, for AD at SCID interview.

Candidates were excluded from study for SCID-verified psychiatric illness: schizophrenia, major depressive disorder, bipolar disorder, posttraumatic stress disorder, or poly-substance dependence (including concurrent antisocial personality disorder). Systemic physical illness excluded those with any liver disease history, bilirubin above 1.2 mg/mL, ALT or AST above 200 U/L, Alcohol Amnestic Syndrome history, HIV seropositivity, history of head injury resulting in loss of consciousness, seizure disorder history including those caused by ethanol withdrawal, blood evidence of folate or vitamin B-12 deficiency, dementia of any type, history of endocrine dysfunction (including Addison's disease, Cushing's disease, or exogenous steroid use within the past 5 years), and any history of genetically based reactions to alcohol use (Asian ancestry with a history of the ethanol flush response). Alcohol-dependent subjects were excluded if withdrawal symptoms at the time of study entry necessitated hospitalization.

#### Time Line

In this study, heavy-drinking subjects were required to provide a negative breath ethanol test administered by the study staff on the day of consent, the first day of the study protocol and again on the day of the first monitored disulfiram ingestion, as well as previous to each subsequent witnessed disulfiram administration. This was to assure (1) informed consent for study entry, that is, the absence of inebriation and (2) subject safety in disulfiram administration. The MRI scan was performed within 3 days of the last reported alcohol use, within 4 days of the consent; breath alcohol testing was not required on the day of MRI scan. No subjects were scanned while obviously inebriated and none were observed in a disulfiram-ethanol reaction on the scan day. The 3-day limit for inclusion was designed to assure MRI study very early in the course of any structural healing processes that might have begun with abstinence. All of the subjects were ambulatory volunteers and none were inpatients at the time of study entry. Control subjects did not receive disulfiram, but reported negative alcohol use histories at the time of study entry (TLFB) and offered no evidence of intoxication.

#### MRI Scan

All entered subjects completed a baseline 3T-MRI brain scan. Scan data were collected through whole brain-volume acquisition using a 3D inversion-recovery spoiled grass (IR-SPGR) pulse sequence in the coronal plane with an image matrix of  $256 \times 192 \times 124$  on a 3T-MRI scanner (General Electric Company, Milwaukee, WI). The image resolution was  $0.94 \times 0.94 \times 1.7 \text{ mm}^3$ , with a lower resolution along the anterior/posterior direction. The inversion time of 450 ms was selected to optimize the gray/white matter contrast. The data acquisition time for the 3D volume was 13 minutes and 11 seconds.

#### Volume Measurement

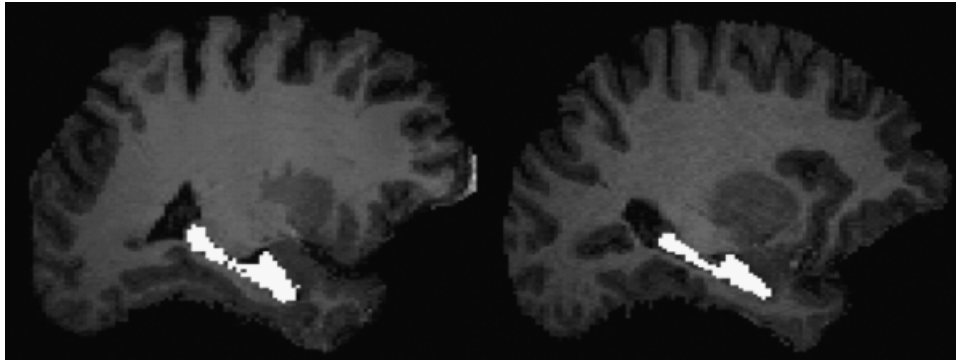
Hippocampal, total brain, and intracranial volumes (ICVs) were derived using an automated segmentation process on the 3T-MRI images of the brain (Shen and Davatzikos, 2002). These were assessed by an imaging analysis research group at the University of

Pennsylvania who analyzed the MRI scan data blind to the subjects' study group membership. The steps in image analysis included (1) removal of extracranial tissues (skull-stripping); (2) tissue segmentation into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF); and (3) elastically warping a labeled atlas to all individual subjects, to label and measure automatically the regions of interest in the brains. These steps are described briefly.

- (1) *Skull stripping*: A seed-based region growing procedure was applied first, which separates the brain parenchyma from extracranial material (Goldszal et al., 1998). Manual editing was then performed on a slice-by-slice basis, which also removes the cerebellum. Comparison with original, unstripped scans at manual editing assures scan data quality for the next step. The interoperator reliability test revealed nonsignificant differences in the manual editing between the 2 trained operators. For 14 image sets evaluated, the mean within-subject difference between raters was  $-0.02 \pm 1.37\%$  for white matter and  $0.46 \pm 0.88\%$  for gray matter. Correlations were greater than 0.99 for both measures. Finally, paired *t*-test comparisons yielded no significant differences between raters.
- (2) *Tissue segmentation*: The SPGR data are segmented into GM, WM, and CSF (Goldszal et al., 1998). An automated segmentation algorithm (Segal et al., 1995) based on *k*-means clustering and Markov random fields, which has been validated extensively (Davatzikos and Resnick, 1998; Goldszal et al., 1998), is used at this step. This method also applies correction for magnetic field inhomogeneities.
- (3) *Automated measurement of brain structures*: A labeled atlas is transformed spatially into spatial coregistration with each tissue-segmented individual brain scan via an elastic warping algorithm (Davatzikos et al., 2001a, 2001b; Shen and Davatzikos, 2002, 2003), referred to as Hierarchical Attribute Matching Mechanism for Elastic Registration (HAMMER), thus obtaining the automatic labeling of brain structures in each subject brain. By calculating the total volume in each structure with identical label, the volumetric measurement for each brain structure in each subject can be obtained. To achieve this, we first adopted a finely parcellated brain image as a labeled atlas developed by Kabani and colleagues at the Montreal Neurological Institute (Kabani et al., 1998), including the hippocampal regions of interest (ROIs). Left and right hippocampi have separate labels in this atlas. We then used a HAMMER registration algorithm (Shen and Davatzikos, 2002, 2003) to warp this atlas to each individual subject's image, and thus obtain the automatic labeling of the ROIs in each subject and further obtain their volumetric measurements by computing the volume in each labeled ROI. When computing the volume in each labeled ROI, all voxels, whether they are gray matter voxels or white matter voxels, were counted. Figure 1 shows the automatically labeled hippocampus in representative control and AD subjects. The accuracy of the HAMMER registration algorithm has been extensively validated by both real data and simulated data (Shen and Davatzikos, 2002, 2003).

#### Statistical Analysis

Scan-derived volumetric measurement data acquired in blinded fashion were analyzed for test or control group membership. Differences in AD test versus non-AD control group means in total (combined), right, and left hippocampal mean volumes, respectively, were compared and assessed for statistical significance using Student's *t*-test of the means for samples with differing variances. Significance was set at 0.05 in a one-tailed test as indicated by the a priori prediction of the direction of mean values. We conducted a secondary analysis using multiple linear regressions to include potential confounding variables. These included total brain volume



**Fig. 1.** Labeled hippocampus in representative subjects: control subject (left) and heavy-drinking subject (right).

(TBV) and ICV measures to assess the possible effects of individual variations in brain and calvarium volumes. In this analysis, TBV is the sum of all white matter, gray matter, and ventricular CSF, while ICV is the total volume inside the skull. If no confounding variables were identified at the 0.1 level of probability, they were removed from analysis. If no variables remained, the model was reduced to a simple *t*-test of the means. As a convergent analysis, we conducted partial correlation coefficient (PCC) tests between THV and TBV and ICV, respectively.

#### Sample Derivation

From a total of 54 screened cases (35 test and 19 control), we accrued a sample of 18 matched subjects who proceeded to MRI scan: 9 AD test and 9 non-AD control. The most common reasons for failing study qualification included stopping drinking more than 3 days before study, medical illness exclusion, and presence of Axis I/II disorders mentioned above. Two subjects, 1 from each group, were removed from final data analysis because of potentially significant anatomic abnormalities on the MRI scans that were not regarded as related to alcohol use and that indicated clinical referral (evidence of previous anoxic injury and congenital malformation, respectively). Both subjects were Caucasians; dropping them did not affect either age or ethnic distribution. Data were analyzed from the remaining 16 subjects, 8 per group.

#### Subjects

Owing to the matching procedure, the mean subject age was equivalent between groups: AD-test group  $47.25 \pm 10.71$  years, non-AD control group  $47.75 \pm 10.78$  years. There were 7 Caucasians and 1 African American in each group. Within the heavy-drinking group, the subjects' reported average number of total drinks in the 30 days before study entry was  $392 \pm 259$  standard drinks; however, the average alone is somewhat misleading. Of the 8 heavy drinkers, 5 drank daily and 3 drank during binges that lasted 3 to 4 days weekly. For perspective, all drank an average of  $16 \pm 7$  standard drinks on those days when they drank.

By contrast, the control subjects reported far less alcohol exposure in the prior 30 days. The 8 of them reported a total intake of 24 standard drinks for the previous 30 days, an average of  $3.0 \pm 3.3$  standard drinks each for the entire month, or about  $0.75 \pm 0.8$  standard drinks per person weekly. The range was 0 to 8 drinks during the month. Of the 8 test subjects, 1 subject met SCID criteria for current cannabis abuse but not dependence; 1 met criteria for current stimulant abuse but not dependence; and 1 met criteria for current cocaine dependence. None of the 8 control subjects met criteria for current dependence or abuse of any substance; only 1 met

criteria for a substance-related diagnosis: past alcohol abuse (the abuse occurred over 30 years before study entry).

## RESULTS

### Volume Measures

As shown in Table 1, the total ( $p = 0.02$ ) and left ( $p = 0.01$ ) mean volumes were significantly smaller in the AD heavy drinkers than in the light-drinking, non-AD controls. The right hippocampus mean difference narrowly missed significance ( $p = 0.051$ ) between groups. When tested independently, mean measures of TBV and ICV each differed significantly between the 2 groups (Student's *t*-test, 2 tails). The mean TBV was  $917.4 \pm 98.1$  mL in the alcohol group versus  $1,040.5 \pm 74.3$  mL in the control group ( $p = 0.014$ ). The mean ICV was  $1,024.4 \pm 104.1$  mL among the drinkers versus  $1,162.1 \pm 96.9$  mL among the control subjects ( $p = 0.016$ ).

### Regression and Correlation Analyses

Because the data sufficiently fit a normal distribution and did not require any transformation, we proceeded to multiple linear regression analysis. Drinking status was forced into the model during backward stepwise regression to assess our primary scientific question at all times. To evaluate whether hippocampus volumes were related to brain or calvarium size, TBV and ICV measures were tested in the regression model as covariates. In the multivariate model for each side of the hippocampi, neither covariate contributed significantly (right hippocampus,  $p = 0.42$ ,  $p = 0.65$ , respectively; left hippocampus,

**Table 1.** Mean Hippocampal Volumes (mean mL  $\pm$  SD)

	AD heavy drinkers ( $n = 8$ )	Non-AD light drinkers ( $n = 8$ )	<i>p</i>
Total	$5.47 \pm 0.61$	$6.49 \pm 1.1$	0.020
Left	$2.94 \pm 0.37$	$3.60 \pm 0.59$	0.010
Right	$2.53 \pm 0.28$	$2.89 \pm 0.50$	0.051

AD, alcohol-dependent.

$p = 0.25$ ,  $p = 0.46$ , respectively). When the total hippocampal volume was considered, neither TBV nor ICV significantly contributed to the model ( $p = 0.31$ ,  $p = 0.53$ , respectively) when drinking status was included. With volume covariates showing no effect, we concluded that Student's  $t$ -test was the appropriate statistic for assessment of between-group hippocampus volumes measures.

Using the whole sample ( $n = 16$ ), we calculated the partial correlation coefficients (PCC) between THV and TBV, as well as THV and ICV, controlling for drinking status as either a heavy or a light drinker. We found no association between THV and either variable: for total brain volume,  $PCC = 0.281$ ,  $p = 0.31$ ; for intracranial volume,  $PCC = 0.175$ ,  $p = 0.53$ .

## DISCUSSION

The data presented here support the hypothesis that chronic, heavy drinking of ethyl alcohol is associated with reduced THV and that observed volume reductions are likely independent of total brain and intracranial volumes. Although the data are derived from a relatively small sample, the subjects represent a group selected to be free of variables previously reported as potentially confounding volumetric MRI data—the exclusion criteria listed above. As a result, we offer these results as clearly implicating an injurious role of chronic heavy ethanol use alone.

For an added perspective, we construed the group mean differences as a drug effect of ethanol. The calculated effect difference in THV between the AD and control groups yielded Cohen's  $d = 1.1$ . For left and right hippocampus volumes,  $d = 1.3$  and  $0.9$ , respectively. Cohen's statistic defines effect sizes as large equaling 0.6 to 0.8 or greater, medium 0.3 to 0.5, and small 0.0 to 0.2 (Cohen, 1988). The large effect size in this case appears best attributed to the difference in drinking status between these 2 groups. As a comparison for discussion purposes, we calculated this statistic from the reported effect data of naltrexone on days abstinent as reported in a recent multicenter trial (Anton et al., 2006). Those data yielded  $d = 0.24$ , only a small effect. In the same study, medical management without the study medication resulted in  $d = 0.49$ , a medium effect. By contrast, the effect of ethanol that we observed in reducing THV appears to be a sizable one.

This study has several limitations that prevent generalization to all heavy, sustained users of alcohol. As mentioned, the data presented from a small and highly select sample gathered to establish that hippocampus volume loss can be reliably observed. The small sample size may have to do with the smaller mean ICV that we observed in the drinking subjects; while previous research strongly suggests lessened mean TBV in heavy drinkers, no reports in our awareness note lessened ICV as a general characteristic in a single gender sample. Concomitantly,

this sample includes no female subjects and some reports suggest that gender may be a confounding factor in any comparison with male and female AD drinkers (Pfefferbaum et al., 2001; Gianoulakis et al., 2003). Other possible variables of interest were not recorded including handedness, socioeconomic status, or body size. Our previous research (Lucey et al., 1999), however, casts doubt as to whether body size is a contributing variable in a design controlling for age and gender. Finally, this study did not address any possible secondary molecular effects from high ethanol exposure (Bleich et al., 2003a).

The test subjects in this study were seen in middle age after long, heavy-drinking careers. Although a recent report found that adolescents with alcohol use disorders who are free of psychiatric comorbidities experience a reduction in the left hippocampus (Nagel et al., 2005), our data offer no comment on heavy, sustained drinking at an earlier age, for example, binge drinking in young adults when the course of heavy drinking is comparatively early.

Future directions suggested by this line of research include enlarging the sample beyond middle-aged, male, DVA subjects in an effort to arrive at more generalizable conclusions. Future replication study should include a wider sampling of heavy drinking men and a large sample of heavy-drinking women. If the data continue to suggest THV lessening, studies at earlier points in the drinking career—such as in heavy-drinking adolescents—as well as in specific minority groups would be indicated. The data observed here relate only a cross-sectional view of THV and raise the importance of recording the natural history of hippocampus volume change, if any, over the course of abstinence from ethanol. While the present report suggests injury to the hippocampus, injuries are often capable of healing in a healthy environment. It is our hope, as well, to explore this in serial, controlled MRI studies of active AD drinkers.

## REFERENCES

- Agartz I, Momenan R, et al (1999) Hippocampal volume in patients with alcohol dependence. *Arch Gen Psych* 56:356–363.
- Anton RF, O'Malley SS, et al (2006) Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study; a randomized controlled trial. *JAMA* 295:2003–2017.
- Beresford T, Arciniegas D, et al (1999) Hippocampal to pituitary volume ratio: a specific measure of reciprocal neuroendocrine alterations in alcohol dependence. *J Stud Alcohol* 60:586–588.
- Bleich S, Bandelow B, et al (2003a) Hyperhomocysteinemia as a new risk factor for brain shrinkage in patients with alcoholism. *Neurosci Lett* 335:179–182.
- Bleich S, Sperling W, et al (2003b) Lack of association between hippocampal volume reduction and first-onset alcohol withdrawal seizure. *Alcohol Alcohol* 38:40–44.
- Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates, Hillsdale, NJ.
- Davatzikos C, Genc A, et al (2001a) Voxel-based morphometry using the RAVENS maps: methods and validation using simulated longitudinal atrophy. *NeuroImage* 14:1361–1369.

- Davatzikos C, Li HH, et al (2001b) Accuracy and sensitivity of detection of activation foci in the brain via statistical parametric mapping: a study using a PET simulator. *Neuroimage* 13:176–184.
- Davatzikos C, Resnick SM (1998) Sex differences in anatomic measures of interhemispheric connectivity: correlations with cognition in men but not in women. *Cereb Cortex* 8:635–640.
- Gianoulakis C, Dai X, et al (2003) Effect of chronic alcohol consumption on the activity of the hypothalamic-pituitary-adrenal axis and pituitary beta-endorphin as a function of alcohol intake, age, and gender. *Alcohol Clin Expi Res* 27:410–423.
- Goldszal AF, Davatzikos C, et al (1998) An image processing protocol for the analysis of MR images from an elderly population. *J Comput Assist Tomograp* 22:827–837.
- Kabani N, MacDonald D, et al (1998) A 3D atlas of the human brain. *Neuroimage* 7:S717.
- Kessler RC, Abelson J, et al (2004) Clinical calibration of DSM-IV diagnoses in the World Mental Health (WMH) version of the World Health Organization (WHO) Composite International Diagnostic Interview (WMHCIDI). *Int J Methods Psychiatric Res* 13:122–139.
- Lucey MR, Hill EM, et al (1999) The influences of age and gender on blood ethanol concentrations in healthy humans. *J Stud Alcohol* 60:103–110.
- Nagel BJ, Schweinsburg AD, et al (2005) Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. *Psychiatry Res* 139:181–190.
- Peters RH, Greenbaum PE, et al (1998) Prevalence of DSM-IV substance abuse and dependence disorders among prison inmates. *Am J Drug Alcohol Abuse* 24:573–587.
- Pfefferbaum A, Rosenbloom M, et al (2001) Sex differences in the effects of alcohol on brain structure. *Am J Psychiatry* 158:188–197.
- Sbrana A, Dell’Osso L, et al (2003) Acceptability, validity and reliability of the Structured Clinical Interview for the Spectrum of Substance Use (SCI-SUBS): a pilot study. *Int J Methods Psychiatri Res* 12:105–115.
- Segal DL, Kabacoff RI, et al (1995) Update on the reliability of diagnosis in older psychiatric outpatients using the structured clinical interview of DSM IIIR. *J Clin Geropsychol* 1:313–321.
- Shen D, Davatzikos C (2002) HAMMER: Hierarchical attribute matching mechanism for elastic registration. *IEEE Trans Med Imaging* 21:1421–1439.
- Shen DG, Davatzikos C (2003) Very high resolution morphometry using mass-preserving deformations and HAMMER elastic registration. *NeuroImage* 18:28–41.
- Sobell LC, Maisto SA, et al (1979) Reliability of alcohol abusers’ self-reports of drinking behavior. *Behav Res Therapy* 17:157–160.
- Sullivan EV, Marsh L, et al (1995) Anterior hippocampal volume deficits in nonamnesic, aging chronic alcoholics. *Alcohol Clin Exp Res* 19:110–122.
- Sullivan EV, Marsh L, et al (1996) Relationship between alcohol withdrawal seizures and temporal lobe white matter volume deficits. *Alcohol Clin Exp Res* 20:348–354.
- Ventura J, Liberman RP, et al (1998) Training and quality assurance with the Structured Clinical Interview for DSM-IV (SCID-I/P). *Psychiat Res* 79:163–173.