

Independent quantitative trait loci influence ventral and dorsal hippocampal volume in recombinant inbred strains of mice

Maureen V. Martin^{†,‡}, Hongxin Dong[†], Dana Vallera[†], David Lee[†], Lu Lu[§], Robert W. Williams[§], Glenn D. Rosen[¶], James M. Cheverud^{**} and John G. Csernansky^{†,**}

[†]Department of Psychiatry, Washington University School of Medicine, [‡]Department of Psychology, St. Louis University, St. Louis, MO, [§]Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Center of Neuroscience, TN, [¶]Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, ^{**}Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO, USA

*Corresponding author: J. G. Csernansky, Department of Psychiatry, Campus Box 8134, Washington University School of Medicine, 660 S. Euclid, St Louis, MO 63110, USA. E-mail: jgc@contc.wustl.edu

Anatomical and functional studies support segregation of the hippocampus into ventral and dorsal components along its septotemporal axis. However, it is unknown whether the development of these two components of the hippocampus is influenced by common or separate genetic factors. In this study, we used recombinant inbred strains of mice to determine whether the same or different quantitative trait loci (QTL) influence ventral and dorsal hippocampal volume. Using two sets of strains of recombinant inbred mice (BXD and AXB/BXA), we identified separate QTLs for ventral and dorsal hippocampal volume. In BXD mice, suggestive QTLs for ventral hippocampus were identified on chromosomes 2, 8 and 13, and a significant QTL for dorsal hippocampal volume was identified on chromosome 15. There was also a suggestive QTL for dorsal hippocampal volume on chromosome 13. In AXB/BXA mice, there were no significant or suggestive QTLs for ventral hippocampal volume, but a significant QTL for dorsal hippocampus was identified on chromosome 5. These findings suggest that the development of the ventral and dorsal components of the hippocampus is influenced by separate genetic loci.

Keywords: Anterior hippocampus, AXB/BXA, BXD, dorsal hippocampus, mice, posterior hippocampus, quantitative trait locus, recombinant inbred strains, schizophrenia, ventral hippocampus

Received 26 June 2005, revised 28 December 2005, accepted for publication 26 January 2006

In mammals, the hippocampus can be separated using functional and anatomical criteria along its septotemporal axis into two components; the ventral or anterior hippocampus and the dorsal or posterior hippocampus (Bannerman *et al.* 2003; Goldman & Mitchell 2004; Moser & Moser 1998; Richmond *et al.* 1999; Trivedi & Coover 2004). In rodents, dorsal hippocampus function has been implicated in spatial memory (Pothuizen *et al.* 2004), while ventral hippocampus function has been implicated in fear and anxiety responses (Degroot & Treit 2004; Trivedi & Coover 2004).

The separation of the hippocampus into ventral and dorsal components is consistent with the topography of its connections with various regions of the cerebral cortex and subcortical structures. In the rodent and primate, axons that originate in the lateral and medial entorhinal cortex terminate mainly at the septal and temporal level of the dentate gyrus and Ammon's horn, respectively (van Groen *et al.* 2003; Witter *et al.* 1989;). There are reciprocal projections from amygdala nuclei to the temporal half of the CA1 subfield (Pitkänen *et al.* 2000). The ventral, but not the dorsal hippocampus, projects to the lateral and medial prefrontal cortex (Verwer *et al.* 1997) and also appears to preferentially project to the medial nucleus accumbens (Friedman *et al.* 2002). Finally, levels of acetylcholinesterase, glutamate, serotonin are higher in the ventral than in the dorsal hippocampus (Hörtnagl *et al.* 1991).

It is unknown whether genetic factors that might influence the development of these two components of the hippocampus are common or separable. To address this question, we used recombinant inbred (RI) strains of mice to map associations between quantitative trait loci (QTL) and ventral and dorsal hippocampal volume. RI strains of mice allow for rapid identification of QTLs, because all individuals within a strain are isogenic, and the genome of each strain has been characterized. Recent studies have used RI mice to associate QTLs with the volume of the entire hippocampus, as well as the volumes of the dentate gyrus and granule cell layer (Peirce *et al.* 2003). Such studies are facilitated by the GeneNetwork (<http://www.genenetwork.org>), which is a program developed for sophisticated QTL mapping and enables the analysis of genetic influences at many levels, including neuroanatomical and behavioral phenotypes and gene expression.

The purpose of the present study was to examine whether there were separable genetic associations for the ventral and

Table 1: QTL for ventral and dorsal hippocampal volume.

Trait	Chr	QTL	Proximal	Distal	Effect Size	Effect Size/SD	LRS	LOD	Support Interval Length	Proximal (Mb)	Distal (Mb)
Ventral											
Hippocampus	2	<i>D2Mit296</i>	<i>D2Mit82</i>	<i>SNP Ass1</i>	1.76	1	9.7	2.1	5.46	25.98	31.44
	8	<i>D8Mit31</i>	<i>D8Mit132</i>	<i>D8Mit75</i>	1.99	1.13	13.1	2.8	16.84	70.32	81.60
	13	<i>SNP 13.067.300</i>	<i>D13Mit94</i>	<i>SNP 13.069.159</i>	2.00	1.14	12.2	2.7	19.05	64.01	66.26
Dorsal											
Hippocampus	13	<i>D13Mit115</i>	<i>D13Mit57</i>	<i>D13Mit60</i>	1.58	0.67	10.5	2.3	18.95	16.37	35.31
	15	<i>D15Mit149</i>	<i>D15Mit147</i>	<i>D15Mit35</i>	1.88	0.80	16.4	3.6	10.69	93.31	104.00
	5*	<i>D5Mit312</i>	<i>D5Mit20</i>	<i>D5Mit201</i>	2.20	1.11	10.5	2.3	21.34	95.19	73.85

Chr, Chromosome; Distal, the most distal marker 1-LOD score drop from the peak QTL; Effect Size, the size of the effect of the QTL; LOD, logarithm of the odds ratio; LRS, likelihood ratio statistic; Proximal, the most proximal marker 1-LOD score drop from the peak QTL; QTL, the marker at the peak of the quantitative trait locus; SD, standard deviation derived from a pooled within genotype estimate; Support Interval Length, length of the QTL interval based upon Build 34 coordinates.

*in AXB/BXA mice, QTLs in BXD mice unless otherwise noted.

dorsal hippocampus by (a) examining the genetic correlation between these phenotypes and (b) determining whether there were common or separate QTLs for the two phenotypic measures within strains in two series of RI mice. In addition, we used the GeneNetwork and archived behavioral data to test for structure-function correlations within strains.

Materials and method

Animals

QTLs were mapped separately using data available from the Mouse Brain Library (MBL) (<http://www.mbl.org>) in two sets of RI mice: BXD and AXB/BXA. A total of 144 BXD recombinant RI mice from 36 strains (including the C57BL/6J and DBA/2J parental strains) were used. In this experiment, the average number of mice per strain was four (range 2–5). The average age was 106 days (range 21–694, SD = 100.7), and there were greater numbers of male ($n = 82$) than female ($n = 62$) mice. A total of 112 AXB/BXA RI mice from 30 different strains (including the C57BL/6J and A/J parental strains) were used in a second experiment. In that experiment, the average number of mice per strain was 3.7 (range 2–7). The average age was 95 days (range 37–365, SD = 73.6), and there were approximately equal numbers of male ($n = 52$) and female ($n = 60$) mice. See Table 2(a) and (b) for age ranges for BXD and AXB/BXA mouse strains.

Volume measurement

Digital images of 30 μm -thick, Nissl-stained mouse brain sections were obtained from the MBL (<http://www.mbl.org>); the procedures for processing tissue have been described previously (Zygorakis & Rosen 2003). Briefly, animals were deeply anesthetized with Avertin (0.5–0.8 ml, i.p.) and transcardially perfused with 0.9% phosphate-buffered saline (PBS) followed by 15 ml of 1.25% glutaraldehyde and 1.0%

paraformaldehyde and then 10–20 ml of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.9% PBS. The head was removed and put in fixative until the brain was dissected. The brains were embedded in 12% celloidin and sectioned in the horizontal plane at 30 μm . Four sets of sections (the first, second, sixth and seventh series) containing every tenth section were stained with cresyl violet. Measurements were made using two sets, and averages for each animal were used in our analyses.

Horizontal section images were formatted, and measurements were made using ANALYZE 7.0 (Biomedical Imaging Resources, Mayo Clinic, Rochester, MN, USA). First, a grid was placed over each section image. Then, all points that intersected with the structure of interest were selected, and the total number of points was counted (Fig. 1). Volumes from the two sets of sections were averaged for each animal, except in five BXD animals and four AXB/BXA animals, where images from only one of the sets were available. Volume estimates for the whole hippocampus and its ventral region were obtained by multiplying the total number of points that intersected the structure of interest by the distance between points (0.685 μm) and the distance between sections (300 μm). To adjust for shrinkage during brain tissue processing, we divided the brain weight by the brain volume and used the resulting ratio as a shrinkage factor to adjust all volume measurements, as previously described (Peirce *et al.* 2003). Dorsal hippocampal volumes were calculated by subtracting the total number of points that intersected the ventral hippocampus from the total number of points that intersected the whole hippocampus. Landmarks associated with the dentate gyrus were used to divide the hippocampus into ventral and dorsal regions. Using horizontally cut sections, the section at which the granule layer of the dentate gyrus was first split into two parts (Fig. 1) was considered to be the first section of the ventral region of the hippocampus. We measured the volume of the entire hippocampus

Table 2a: Range for BXD mice. The age ranges of animals representing each strain are listed. Strain means for ventral and dorsal hippocampal volume (mm³) before and after age 'brain volume minus region of interest volume' adjustment

Strain	Age Range	Ventral ¹	Ventral ²	Ventral ³	Dorsal ¹	Dorsal ²	Dorsal ³	D2Mit296	D8Mit31	D13Mit115	13.067.300	D15Mit149
BXD1	All 53	20.3 ± 2.2	4.8 ± 2.2	4.8 ± 2.2	14.4 ± 2.8	1.8 ± 2.9	1.9 ± 2.7	D	D	D	B	D
BXD2	38-76	13.0 ± 1.8	-0.5 ± 1.8	-2.4 ± 1.8	11.6 ± 2.3	-1.0 ± 2.5	-0.9 ± 2.4	B	B	B	D	D
BXD5	56-329	17.0 ± 2.3	1.0 ± 1.8	1.1 ± 1.8	14.9 ± 3.0	1.1 ± 2.0	1.2 ± 2.1	D	D	B	B	D
BXD6	34-110	13.1 ± 2.7	-2.5 ± 2.5	-2.6 ± 2.5	13.1 ± 1.4	0.5 ± 1.8	0.3 ± 1.8	B	B	B	D	D
BXD8	59-694	17.9 ± 0.8	0.4 ± 2.4	0.4 ± 2.5	14.5 ± 1.4	0.3 ± 1.5	0.3 ± 1.6	D	D	B	B	B
BXD9	54-185	15.7 ± 2.0	-0.1 ± 2.2	0.1 ± 2.1	14.0 ± 2.6	0.7 ± 2.8	0.7 ± 2.7	D	D	B	D	B
BXD11	49-106	16.1 ± 2.4	0.5 ± 2.4	0.6 ± 2.3	15.6 ± 2.6	2.7 ± 2.8	2.7 ± 2.8	B	D	B	D	D
BXD12	41-108	14.1 ± 1.5	-1.6 ± 1.3	-1.5 ± 1.4	11.5 ± 1.3	-1.4 ± 0.6	-1.3 ± 0.7	B	B	D	D	B
BXD13	35-93	14.8 ± 0.7	-0.8 ± 0.8	-0.8 ± 0.8	11.7 ± 2.5	-1.0 ± 2.3	-1.0 ± 2.4	D	D	D	D	B
BXD14	21-135	14.8 ± 2.3	-0.9 ± 2.1	-0.9 ± 2.3	12.7 ± 3.1	-0.4 ± 2.8	-0.3 ± 2.9	D	B	D	D	D
BXD15	61-182	17.3 ± 1.9	1.5 ± 2.3	1.6 ± 2.2	15.6 ± 1.4	2.1 ± 0.3	2.2 ± 0.3	B	B	B	B	D
BXD16	36-96	15.2 ± 1.5	-0.4 ± 1.4	-0.4 ± 1.4	13.4 ± 2.1	0.7 ± 2.3	0.7 ± 2.3	D	D	B	D	D
BXD18	60-223	14.6 ± 2.0	-1.2 ± 2.0	-1.2 ± 2.0	9.0 ± 6.0	-4.5 ± 5.4	-4.4 ± 5.4	B	D	D	B	B
BXD19	44-109	19.2 ± 3.3	3.6 ± 3.2	3.5 ± 3.1	15.7 ± 5.2	2.7 ± 4.9	2.7 ± 4.8	D	D	B	B	D
BXD20	55-141	15.1 ± 2.0	-0.6 ± 2.2	-0.6 ± 2.2	12.9 ± 0.9	-0.5 ± 1.2	-0.5 ± 1.2	B	B	B	D	B
BXD22	41-184	19.4 ± 0.9	3.6 ± 0.5	3.6 ± 0.6	14.6 ± 2.1	1.0 ± 1.1	1.0 ± 1.1	D	D	B	B	D
BXD23	41-246	16.0 ± 1.5	0.1 ± 1.6	0.1 ± 1.6	13.5 ± 2.4	-0.1 ± 1.6	-0.1 ± 1.5	D	B	D	D	D
BXD24	92-163	15.9 ± 0.5	0.1 ± 0.4	0.1 ± 0.4	12.5 ± 0.5	-1.1 ± 0.8	-1.2 ± 0.8	D	B	B	D	B
BXD25	56-108	14.6 ± 1.2	-1.0 ± 1.2	-1.1 ± 1.1	12.0 ± 2.3	-0.9 ± 2.0	-0.9 ± 2.1	D	B	D	D	B
BXD27	59-234	12.3 ± 1.3	-3.4 ± 1.5	-3.5 ± 1.5	11.5 ± 1.3	-1.9 ± 1.5	-2.1 ± 1.5	B	B	D	D	B
BXD28	70-109	14.0 ± 1.4	-1.7 ± 1.6	-1.7 ± 1.5	13.3 ± 0.8	0.2 ± 1.2	0.2 ± 1.2	B	B	B	D	D
BXD29	52-60	14.7 ± 3.0	-0.8 ± 3.0	-0.9 ± 2.9	10.3 ± 1.4	-2.4 ± 1.4	-2.5 ± 1.4	B	B	D	B	B
BXD30	53-190	14.7 ± 0.9	-1.0 ± 1.1	-1.1 ± 1.0	11.4 ± 0.8	-2.0 ± 1.2	-2.0 ± 1.2	D	B	D	D	B
BXD31	50-128	15.1 ± 1.6	-0.6 ± 1.5	-0.6 ± 1.6	12.2 ± 3.3	-0.9 ± 3.0	-0.9 ± 2.9	D	D	B	D	D
BXD32	47-298	18.2 ± 2.4	3.3 ± 1.3	3.3 ± 1.3	14.1 ± 1.4	0.5 ± 1.2	0.4 ± 1.2	D	D	D	D	B
BXD33	61-64	13.6 ± 1.7	-2.0 ± 1.7	-1.9 ± 1.7	13.6 ± 1.7	0.8 ± 1.7	1.0 ± 1.6	B	B	B	D	D
BXD34	54-79	15.0 ± 0.9	-0.6 ± 0.8	-0.7 ± 0.9	12.8 ± 1.5	0.1 ± 1.6	0.0 ± 1.6	B	D	B	B	B
BXD35	52-56	14.3 ± 0.8	-1.3 ± 0.8	-1.3 ± 0.8	12.2 ± 0.8	-0.4 ± 0.8	-0.4 ± 0.8	B	B	D	D	B
BXD36	51-281	15.7 ± 1.3	-0.1 ± 1.4	-0.1 ± 1.4	12.6 ± 1.1	-0.6 ± 1.2	-0.6 ± 1.3	D	B	D	D	B
BXD38	48-75	16.1 ± 0.0	0.6 ± 0.3	0.5 ± 0.4	12.4 ± 1.0	-0.3 ± 1.0	-0.3 ± 1.0	D	B	B	B	D
BXD39	40-60	14.6 ± 2.1	-0.9 ± 2.1	-1.0 ± 2.1	12.3 ± 1.2	-0.2 ± 1.2	-0.3 ± 1.2	B	D	D	D	D
BXD40	44-58	17.5 ± 1.2	2.0 ± 1.2	2.0 ± 1.2	15.6 ± 2.8	3.1 ± 2.7	3.1 ± 2.7	D	D	B	B	D
BXD42	42-67	16.0 ± 2.1	0.5 ± 2.1	0.5 ± 2.1	13.9 ± 2.5	1.3 ± 2.5	1.3 ± 2.5	D	D	B	B	D
C57	51-316	17.2 ± 1.5	0.8 ± 1.6	0.9 ± 1.6	16.3 ± 0.9	1.6 ± 0.7	1.7 ± 0.7	B	B	B	B	D
DBA	76-294	16.6 ± 0.5	0.3 ± 0.9	0.3 ± 0.9	14.1 ± 1.6	-0.5 ± 1.4	-0.6 ± 1.4	D	D	D	D	B

Ventral¹, Ventral hippocampal strain means and standard deviations before age and 'brain volume minus region of interest' adjustment. Ventral², Ventral hippocampal strain means and standard deviations after adjusting for age related variance using age and/or age². Ventral³, Ventral hippocampal strain means and standard deviations after adjusting for age related variance using age and/or age² and 'brain volume minus region of interest volume'. Dorsal¹, Dorsal hippocampal strain means and standard deviations before age and 'brain volume minus region of interest' adjustment. Dorsal², Dorsal hippocampal strain means and standard deviations after adjusting for age-related variance using age and/or age². Dorsal³, Dorsal hippocampal strain means and standard deviations after adjusting for age related variance using age and/or age² and 'brain volume minus region of interest volume'. Genotype at QTL peak markers are given for each strain. B represents the C57BL/6 J genotype. D represents the DBA/2 J genotype. A '.', '.' signifies that the genotype is unknown. Genotypes were obtained from the GeneNetwork.

Table 2b: Range for AXB/BXA mice. The age ranges of animals representing each strain are listed. Strain means for ventral and dorsal hippocampal volume (mm³) before and after age 'brain volume minus region of interest volume' adjustment

Strain	Age Range	Ventral ¹	Ventral ³	Dorsal ¹	Dorsal ²	Dorsal ³	D5Mit321
AXB1	44–155	15.4 ± 0.7	−0.9 ± 0.7	13.3 ± 3.1	−0.6 ± 2.1	−0.5 ± 2.0	D
AXB2	48–182	16.2 ± 0.5	−0.3 ± 0.4	14.7 ± 2.8	0.1 ± 2.0	0.2 ± 2.1	D
AXB4	126–126	18.5 ± 2.4	2.5 ± 2.3	17.0 ± 0.1	2.2 ± 0.1	1.7 ± 0.1	D
AXB5	40–137	14.2 ± 1.8	−1.9 ± 1.6	13.1 ± 2.3	−0.1 ± 2.5	−0.1 ± 2.6	B
AXB6	39–67	17.5 ± 1.1	1.5 ± 1.1	19.1 ± 1.8	6.0 ± 1.2	5.9 ± 1.0	D
AXB8	43–127	14.8 ± 2.1	−1.1 ± 2.1	13.5 ± 1.4	0.1 ± 1.4	−0.2 ± 1.2	D
AXB10	47–155	17.0 ± 0.2	0.8 ± 0.6	11.8 ± 0.8	−1.8 ± 1.1	−1.8 ± 0.9	B
AXB12	46–196	15.6 ± 1.4	0.5 ± 1.1	13.1 ± 2.0	−1.0 ± 1.1	−1.3 ± 1.2	B
AXB13	74–150	16.9 ± 2.6	0.6 ± 2.3	12.9 ± 4.0	−1.5 ± 3.2	−1.6 ± 3.4	–
AXB15	39–85	16.8 ± 1.7	0.6 ± 1.7	10.9 ± 0.4	−2.0 ± 0.7	−1.8 ± 0.7	B
AXB18	46–605	16.0 ± 1.4	−0.5 ± 1.5	12.9 ± 1.7	−1.3 ± 0.5	−1.2 ± 0.4	–
AXB19	41–114	16.3 ± 1.9	0.1 ± 2.0	10.9 ± 2.0	0.9 ± 1.2	0.9 ± 1.1	–
AXB20	42–222	17.3 ± 2.6	0.7 ± 2.8	12.9 ± 7.0	1.9 ± 1.4	2.0 ± 1.3	–
AXB24	39–140	13.3 ± 1.5	−2.9 ± 1.0	16.6 ± 2.4	−1.0 ± 0.7	−1.0 ± 0.8	B
BXA1	43–87	16.3 ± 0.9	0.3 ± 0.7	12.8 ± 2.3	−0.3 ± 2.3	−0.3 ± 2.3	B
BXA2	48–102	18.2 ± 2.3	2.0 ± 2.3	13.2 ± 2.1	0.0 ± 1.7	0.1 ± 1.7	B
BXA4	49–191	15.4 ± 1.0	−0.5 ± 1.3	14.2 ± 1.6	0.0 ± 2.0	−0.5 ± 1.9	B
BXA7	39–172	18.0 ± 1.3	2.0 ± 1.6	13.7 ± 1.5	0.1 ± 1.3	0.0 ± 1.1	D
BXA8	40–116	15.7 ± 1.1	0.4 ± 0.9	12.8 ± 1.1	−0.4 ± 1.2	−0.4 ± 1.1	B
BXA11	40–92	17.2 ± 3.0	0.8 ± 2.9	14.1 ± 1.1	0.8 ± 1.3	1.1 ± 1.1	B
BXA12	42–182	17.3 ± 2.2	1.0 ± 2.0	14.6 ± 3.4	0.6 ± 2.1	0.8 ± 1.9	B
BXA13	41–147	14.8 ± 5.8	−1.4 ± 6.2	16.7 ± 5.2	3.3 ± 4.2	3.3 ± 4.3	D
BXA14	37–133	14.4 ± 1.5	−1.7 ± 1.3	13.6 ± 1.5	0.0 ± 2.1	−0.1 ± 1.9	B
BXA16	41–116	15.9 ± 1.0	−0.1 ± 0.7	11.4 ± 1.4	−1.9 ± 1.7	−2.0 ± 1.6	B
BXA17	44–144	18.0 ± 1.8	1.6 ± 1.8	13.9 ± 3.4	−0.1 ± 3.0	0.1 ± 2.7	B
BXA24	47–134	14.8 ± 1.9	−1.2 ± 1.6	11.1 ± 0.5	−2.3 ± 0.9	−2.5 ± 0.8	B
BXA25	58–133	16.6 ± 3.6	0.2 ± 3.5	13.8 ± 2.8	0.3 ± 3.3	0.6 ± 3.1	D
BXA26	43–154	14.8 ± 0.7	−1.2 ± 1.0	13.5 ± 0.6	0.0 ± 1.9	−0.2 ± 1.4	B
C57	51–316	17.2 ± 1.5	0.1 ± 1.6	16.3 ± 0.9	1.0 ± 6.0	1.3 ± 0.7	D
AJ	303–365	17.1 ± 1.1	0.1 ± 1.0	14.4 ± 1.7	−1.0 ± 2.2	−1.4 ± 2.1	B

Ventral¹, Ventral hippocampal strain means and standard deviations before age and 'brain volume minus region of interest' adjustment. Ventral², Ventral hippocampal strain means and standard deviations after adjusting for age-related variance using age and/or age². Ventral³, Ventral hippocampal strain means and standard deviations after adjusting for age related variance using age and/or age² and 'brain volume minus region of interest volume'. Dorsal¹, Dorsal hippocampal strain means and standard deviations before age and 'brain volume minus region of interest' adjustment. Dorsal², Dorsal hippocampal strain means and standard deviations after adjusting for age-related variance using age and/or age². Dorsal³, Dorsal hippocampal strain means and standard deviations after adjusting for age related variance using age and/or age² and 'brain volume minus region of interest volume'. Genotype at QTL peak markers are given for each strain. B represents the C57BL/6 J genotype. D represents the DBA/2 J genotype. A '–' signifies that the genotype is unknown. Genotypes were obtained from the GeneNetwork.

formation, including the dentate gyrus, cornu ammonis, fimbria and subiculum.

Data analyses

Backward elimination multiple regression was used to determine whether age, age² (to account for non-linearity), sex, and the variable 'total brain volume minus region of interest (i.e. ventral, dorsal or whole hippocampal) volume' predicted hippocampal volume. Neither sex nor 'total brain volume minus region of interest volume' predicted hippocampal volume. Age and age² predicted BXD dorsal and AXB/BXA dorsal and whole hippocampal volume. Age predicted BXD ventral and

whole. Age did not predict AXB/BXA ventral hippocampal volume. When age and/or age² predicted volume, linear regression was performed to remove age-related variance.

Using the GeneNetwork, marker regression analyses were performed. One-way ANOVAs were run using 781 microsatellite markers and BXD strain means and 668 microsatellite markers and AXB/BXA strain means. To assess genome-wide significance levels, we compared each peak likelihood ratio statistic (LRS) to 5% threshold LRS values computed from 10 000 permutations. In the BXD and AXB/BXA strain sets, six sets of analyses were run, one for each variable: dorsal, ventral and whole hippocampal volume. Age- and

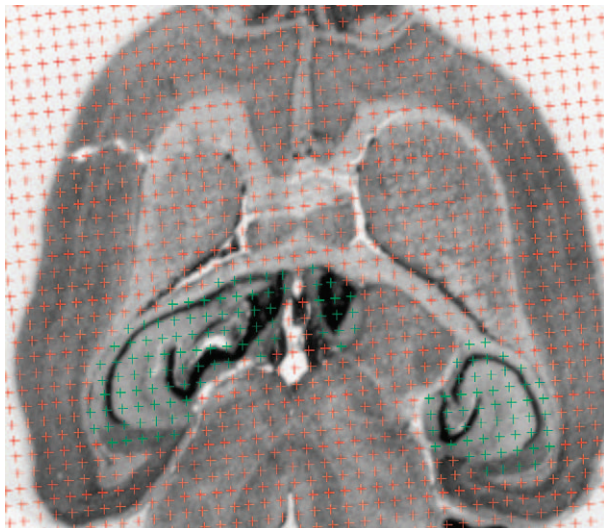


Figure 1: Point counting using ANALYZE 7.0. The grid overlays the brain, and grid points are over the dorsal (on the left) and ventral (on the right); hippocampus points over the hippocampus are totaled. Using the dentate gyrus as a landmark, the first ventral hippocampus section began at the level where the dentate gyrus split into two parts.

age²-corrected residuals were used when appropriate. (See Table 3 for standardized regression coefficients). In a separate set of six analyses, we first forced the variable ‘brain volume minus region of interest volume’ into the regression equation. This was done to determine whether the QTLs identified were related to hippocampal volume via an overall

influence of the QTL on whole brain volume. For each variable, a regression analysis was run using one or more of the following variables: age, age², or brain volume minus region of interest volume. (See Table 3 for standardized regression coefficients and Table 3 for tolerances).

As an exploratory analysis, we selected archival behavioral phenotypes available on the GeneNetwork which were associated with each of the neuroanatomical variables in both sets of RI strains. We selected behavioral phenotypes for this analysis that have been previously associated with hippocampal structure in rodents (Bannerman *et al.* 2004; Deacon & Rawlins 2005; Kusljic & van den Buuse 2004).

Results

Age and sex effects

Using backward linear regression, we examined the effects of sex, age, age² and ‘brain volume minus region of interest volume’ on dorsal, ventral and whole hippocampal volume. Sex did not significantly predict ventral, dorsal or whole hippocampal volume in BXD or AXB/BXA mice. In BXD mice, age, age² significantly predicted dorsal, but not ventral or whole hippocampal volume. Age alone predicted ventral and whole hippocampal volume. Thus, the three hippocampal volume measures were adjusted for age-related effects (See Table 3 for standardized regression coefficients). In AXB mice, age² significantly predicted dorsal hippocampal volume. Neither age nor age² significantly predicted ventral hippocampal volume. Therefore, we adjusted for age-related effects on AXB/BXA dorsal and whole hippocampal volume using linear regression. (See Table 3 for standardized

Table 3: Standardized regression coefficients and tolerances for each independent variable are shown for BXD and AXB/BXA dorsal, ventral and whole hippocampal volume. Two analyses were conducted for each dependent variable. In the first analysis, only age corrections were performed using multiple regression. In the second analysis, age and ‘brain volume minus region of interest volume’ corrections were performed using multiple regression.

Trait	Analysis 1		Analysis 2		
	Age	Age ²	Age	Age ²	Brain minus ROI
Standardized Regression Coefficients					
BXD – ventral	0.19	*	0.20	*	–0.04
BXD – dorsal	0.66	–0.41	0.69	–0.44	–0.05
BXD – whole	0.30	*	0.31	*	–0.06
AXB/BXA – ventral	*	*	0.11	*	0.12
AXB/BXA – dorsal	1.04	–0.65	1.13	–0.70	–0.12
AXB/BXA – whole	1.01	–0.66	1.03	–0.66	–0.03
Tolerances					
BXD – ventral	1.00	*	0.96	*	0.96
BXD – dorsal	0.16	0.16	0.15	0.15	0.96
BXD – whole	1.00	*	0.97	*	0.97
AXB/BXA – ventral	*	*	*	*	1.00
AXB/BXA – dorsal	0.08	0.08	0.08	0.08	0.82
AXB/BXA – whole	0.08	0.08	0.08	0.08	0.83

*The independent variable did not predict volume.

regression coefficients). Strain mean ages were highly variable, and therefore it is possible that by removing age-related variance, we may have also removed some strain-related variance, thus limiting our power to detect weaker QTLs.

Heritability of hippocampal volume measures

Although dominance genetic variance cannot be distinguished from additive and interactive genetic variance in this population, dominance variance is a component in the F₂ strains from which the RI strains are derived. Therefore, we refer to the heritability as the broad-sense heritability. Broad-sense heritability was calculated using age-corrected (when appropriate) and shrinkage-corrected volumes. Heritability was calculated using an R² statistic (Falconer & Mackay 1997; Sokal & Rohlf 1994) as follows:

$$H^2 = C / (MS_{\text{Strain}} + MS_{\text{Error}})$$

where $C = (MS_{\text{Strain}} / MS_{\text{Error}}) / n$

In BXD mice, heritability of whole hippocampal volume, ventral hippocampal volume and dorsal hippocampal volume were 41 (SE = 0.091), 43 (SE = 0.094) and 18% (SE = 0.087), respectively. In AXB/BXA mice, heritability of the whole hippocampal volume, ventral hippocampal volume and dorsal hippocampal volume were 24 (SE = 0.104), 7 (SE = 0.092) and 22 (SE = 0.321), respectively. There are no significant differences in heritability for the same traits in the two RI strain sets.

Genetic correlation between ventral and dorsal hippocampal volume

The genetic correlations between ventral and dorsal hippocampal volume were calculated for BXD and AXB/BXA mice using the shrinkage- and the age-corrected (using age or age-squared, where appropriate) data for ventral and dorsal hippocampal volumes. Genetic correlation was calculated as follows:

$$R(G) = \text{cov}(VH, DH) / \sqrt{V(VH) * V(DH)}$$

where VH indicates ventral hippocampus and DH indicates dorsal hippocampus (Falconer & Mackay 1997).

The genetic correlation between the ventral and dorsal hippocampal volumes in BXD mice was 0.47 ($P < 0.01$, SE = 0.08). The genetic correlation between the ventral and dorsal hippocampal volumes in AXB/BXA mice was 0.03 ($P > 0.05$, SE = 0.09). The genetic correlation between ventral and dorsal hippocampal volume was significantly higher in the BXD mice than in the AXB/BXA mice.

QTL mapping – BXD strains

A suggestive QTL was identified for ventral hippocampal volume on chromosome 8 between markers *D8Mit132* and *D8Mit75*, which accounted for 15.9% of the variance in ventral hippocampal volume (Table 1). The LRS and logarithm of the odds ratio (LOD) score at the peak of this QTL

were 13.1 and 2.8 ($P = 0.001$), respectively. A second suggestive QTL was identified on chromosome 13 between markers *D13Mit94* and SNP *13.069.159*, which accounted for 25.0% of variance in ventral hippocampal volume (Table 1). The LRS and LOD scores at the peak of this QTL were 10.471 and 2.3 ($P = 0.005$), respectively. A third suggestive QTL was identified on chromosome 2, between markers *D2Mit82* and *Ass1*, which accounted for 12.4% of the variance in ventral hippocampal volume (Table 1). The LRS and LOD scores at the peak of this QTL were 9.7 and 2.1 ($P = 0.008$). However, a bootstrap test revealed low reliability of the QTL on chromosome 2. Correlating all of the significant and suggestive markers within the QTL regions revealed a significant correlation between the markers within the QTLs on chromosomes 8 (*D8Mit31*) and 2 (*D2Mit365*) ($r = 0.60$). Correlations above 0.51 were considered large enough to result in 'shadow' QTLs among non-syntenic locations (Cheverud *et al.* 2004; Williams *et al.* 2001). This level of correlation was obtained by multiplying the standard deviation of the random distribution of correlations around the mean of zero, or square root of $(1/\text{total number of strains} - 1)/3$.

To examine the independent effects of markers *D2Mit365* and *D8Mit31*, we searched for QTL effects in subsets of strains with either the C57BL/6J or DBA/2J alleles at each of the markers. We first examined the effects of genotype at marker *D8Mit31* after controlling for genotype at marker *D2Mit365*. In mice with the C57 genotype at marker *D2Mit365*, there was a trend toward an effect of genotype at marker *D8Mit31* on ventral hippocampal volume ($F = 3.77$; $df = 1, 70$; $P = 0.06$). In mice with the DBA genotype at marker *D2Mit365*, there was a significant effect of genotype at marker *D8Mit31* on ventral hippocampal volume ($F = 5.71$; $df = 1, 69$; $P < 0.01$). We then examined the effects of genotype at marker *D2Mit365* after controlling for the effects of genotype at marker *D8Mit31*. In mice with the C57 genotype at marker *D8Mit31*, there was a significant effect of genotype at the *D2Mit365* marker on ventral hippocampal volume ($F = 5.03$; $df = 1, 63$; $P < 0.05$). In mice with the DBA genotype at marker *D8Mit31*, there was a significant effect of genotype at the *D2Mit365* marker on ventral hippocampal volume ($F = 8.25$; $df = 1, 76$; $P = 0.005$). There was no significant interaction effect between genotype at markers *D2Mit365* and *D8Mit31* on ventral hippocampal volume ($F = 0.16$; $df = 3, 140$; $P > 0.05$). The data suggest that both QTLs have independent effects on ventral hippocampal volume.

Dorsal hippocampal volume was associated with a QTL on chromosome 13, between marker *D13Mit57* and *D13Mit60*, which accounted for 19.1% of the variance in dorsal hippocampal volume (Table 1). The LRS and LOD scores at the peak of this QTL were 10.5 and 2.3 ($P = 0.005$), respectively. This appeared to be a separate QTL from the QTL associated with ventral hippocampal volume on chromosome 13. A significant QTL for dorsal hippocampal volume was

also seen on chromosome 15, between markers *D15Mit147* and *D15Mit35*, which accounted for 10.6% of the variance in dorsal hippocampal volume (Table 1). The LRS and LOD scores at the peak of this QTL were 16.4 and 3.6 ($P = 0.0003$), respectively. Finally, there were QTLs for whole hippocampal volume on chromosomes 8, 13 and 15 that overlapped with QTLs for both ventral and dorsal hippocampal volume. When variance associated with 'whole brain volume minus region of interest volume' was removed, all QTLs remained at the suggestive level, and no new QTLs were identified.

QTL Mapping – AXB/BXA strains

A significant QTL was identified for dorsal hippocampal volume on chromosome 5 between markers *D5Mit20* and *D5Mit201*, which accounted for 15.1% of the variance in dorsal hippocampal volume (Table 1). The LRS and LOD scores at the peak of this QTL were 13.5 and 2.9 ($P = 0.001$), respectively. No significant or suggestive QTLs were identified for ventral hippocampal volume. Whole hippocampal volume was also associated with the dorsal hippocampal volume QTL. When variance associated with 'whole brain volume minus region of interest volume' was removed, the QTL remained at the suggestive level, and no new QTLs were identified.

Marker genotype interaction effects

We examined whether the sex or age of the mice influenced the gene effect on hippocampal volume. Although age-corrected strain means were used in other QTL analyses, uncorrected individual volumes were used for this analysis and tested against suggestive and significant QTL to determine whether the QTL effects were confounded with age-related variation. We found a significant interaction effect between BXD ventral hippocampal volume, QTL marker *D8Mit31* and age ($F = 3.93$; $df = 12$; $P = 0.0003$), as well as a significant interaction effect between BXD ventral hippocampal volume, QTL marker rs08.076.440 and age ($F = 4.66$; $df = 13$; $P < 0.0001$), on dorsal hippocampal volume. Further, the effect of age on dorsal hippocampal volume in animals with C57BL/6J and DBA/2J genotypes at these markers predicted dorsal hippocampal volume in animals with the C57BL/6J genotype at the *D8Mit31* marker ($F = 25.48$; $df = 1, 62$; $P < 0.0001$). These data suggest that the age of the mice influenced the gene effect on volume. However, the animals were not evenly distributed within strains in terms of age or sex, and thus genotype and age/sex were not independent variables.

Marker regression was run using non-age-adjusted ventral and dorsal hippocampal volumes to determine whether the age-adjustment biased our results. Using non-age-adjusted BXD ventral hippocampal volumes, a significant QTL was found on chromosome 8, and a suggestive QTL was found on chromosome 13. Using non-age-adjusted

BXD dorsal hippocampal volumes, a suggestive QTL was found on chromosome 15. Using non-age-adjusted AXB/BXA dorsal hippocampal volumes, a suggestive QTL was found on chromosome 5. These results suggest that age adjustments did not create false QTLs.

Behavioral correlates

As an exploratory analysis, we correlated volume measurements for the strains with archival behavioral data previously associated with hippocampal structure across strains using the GeneNetwork. The selected behaviors included spatial memory (Morris water maze performance), fear conditioning, exploration during the light-dark paradigm, exploratory locomotor activity and sensorimotor gating. In BXD mice, ventral hippocampal volume was inversely correlated with performance on the Morris water maze performance ($n = 10$; $r = -0.63$; $P = 0.049$), a test of spatial memory, and positively correlated with the response to acoustic startle ($n = 25$; $r = 0.48$; $P = 0.01$). Dorsal hippocampal volume was also inversely correlated to Morris water maze performance, although not significantly ($n = 10$; $r = -0.6$; $P = 0.07$). QTL mapping of these behavioral traits did not suggest overlap between structural and behavioral QTLs, although acoustic startle response was associated with genotype at marker *D2Mit296* at the peak of the ventral hippocampus QTL. Examination of the scatterplots from these correlations did not reveal outliers that may have biased these results.

Next, we specifically examined the correlations between genotype of markers at the peaks of our QTLs and behaviors that have been linked to ventral and dorsal hippocampus function. A negative correlation signifies a positive relationship between the C57BL/6J genotype and performance on the behavioral measure. Genotype at marker *D2Mit296* was correlated to open-field activity ($n = 22$; $r = -0.53$; $P = 0.01$) and acoustic startle response to 10 kHz white noise bursts ($n = 26$; $r = -0.42$; $P = 0.03$). Genotype at marker *D5Mit312* was correlated to number of transitions in the light/dark paradigm ($n = 26$; $r = -0.40$; $P = 0.04$), and latency to enter a novel side in a test of exploratory locomotor activity ($n = 12$; $r = 0.59$; $P = 0.04$). However, there were two outliers in the exploratory locomotor activity dataset, which may have biased the result in this small sample. Spatial memory did not correlate with any of the hippocampal volume QTL peak markers.

Finally, we also examined the relationship between dorsal-to-ventral hippocampal volume ratio and behavior in BXD and AXB/BXA strains. We found an inverse correlation between larger dorsal-to-ventral hippocampal volume ratio and more pronounced prepulse inhibition of the acoustic startle response ($n = 24$; $r = -0.53$, $P = 0.006$), locomotor activity ($n = 26$; $r = -0.49$, $P = 0.01$) and acoustic startle response ($n = 25$; $r = -0.44$, $P = 0.03$) in BXD mice. Examination of the scatterplots showed

outliers were not at play for these datasets. There were no correlations between dorsal-to-ventral hippocampal volume and hippocampal-dependent behaviors in AXB/BXA mice, using the less extensive archival data available for these strains compared with BXD mice.

Discussion

The results of this study demonstrate that there are separate QTLs for ventral and dorsal hippocampal volume in two different RI strain sets. In BXD mice, there were suggestive QTLs for ventral hippocampal volume on chromosomes 2, 8 and 13, and a significant QTL for dorsal hippocampal volume on chromosome 15. There was also a suggestive QTL for dorsal hippocampal volume on chromosome 13 in a different location from the ventral hippocampus QTL on that chromosome. In AXB/BXA mice, we identified a significant QTL for dorsal hippocampal volume on chromosome 5, and no QTLs for ventral hippocampal volume. All QTLs remained after correcting for 'brain volume minus region of interest volume' suggesting that these QTLs do not influence hippocampal volume solely through their influence on whole brain volume.

The genetic correlation between ventral and dorsal hippocampal volume in BXD mice was 0.47. Thus, ventral and dorsal hippocampal QTLs would be expected to overlap at 22% (or $\sqrt{0.47}$) of the QTLs. We did not have sufficient power to detect QTLs that may have been responsible for this genetic correlation. In AXB/BXA mice, the genetic correlation was 0.03, which supports the notion that genetic factors influencing ventral and dorsal hippocampal volume are independent in these strains.

These results replicate and extend previous work studying genetic influences on whole hippocampal volume and structure in BXD mice (Peirce *et al.* 2003). These authors also used BXD RI mice and reported a QTL on chromosome 13 that influenced the volume of the dentate gyrus of the hippocampus and a QTL on chromosome 15 that influenced the volume of the whole hippocampus. In the latter case, the QTL that was associated the volume of the whole hippocampus was in the same region as the one found to be associated with dorsal hippocampal volume in this study. These authors also reported a QTL associated with a region on chromosome 6; however, we did not find linkage to this region in our study. Discrepancies between the results of this study and the work of Peirce and colleagues could be related to differences in neuroanatomical definitions, the methods used for volume measurement or sample size. For example, we made our measurements in the horizontal plane rather than measuring both in the horizontal and coronal planes, as was done by Peirce *et al.* (2003), and we did not measure volume in the coronal plane, which may have reduced our ability to detect subtle volume differences along the anterior–posterior axis. In addition, we included the subiculum within the volume of the hippocampus, so that our

results could be more relevant to magnetic resonance imaging studies in humans, where the subiculum usually is included as part of the hippocampus (Csernansky *et al.* 2002). Nonetheless, the correlation between our measures of whole hippocampal volumes and the volumes reported by Peirce *et al.* (2003) was $r = 0.77$, $P = 1.6 \times 10^{-8}$ ($n = 34$).

We also found an age-related increase in ventral and whole hippocampal volume and a curvilinear relationship between age and dorsal hippocampal volume in the BXD strain set. A curvilinear relationship between age and dorsal and whole hippocampal volume was also detected in the AXB/BXA strain set. These findings suggest an interaction between genotype and age with respect to ventral hippocampal volume. There was also an interaction between genotype at the *D8Mit31* marker and age on dorsal hippocampal volume in the BXD strain set suggesting the effects of a gene near this marker on volume varies with age. Interestingly, the relationship between age and hippocampus in humans is complex. The process of myelination of fibers within the human hippocampal complex begins at birth and continues to increase into adulthood (Arnold & Trojanowski 1996). Thus, the volume of the hippocampus increases at a rapid pace until 2 years of age, and more slowly thereafter into adulthood (Utsunomiya *et al.* 1999). Myelination continues to occur within the subiculum and presubiculum throughout adulthood (Benes *et al.* 1994). The volume of the hippocampus does not appear to decrease substantially with age in middle-aged adults (Sullivan *et al.* 2005). However, decreases in hippocampal volume may be associated with aging in the elderly (Szentkuti *et al.* 2004). Our finding of a curvilinear relationship between age and dorsal, but not ventral, hippocampal volume in both BXD and AXB/BXA mice suggests that the dorsal hippocampus may be more affected by age-related volume loss.

The parental strains, C57BL/6, DBA/2 and A/J, which were used to generate the RI strain sets used in these experiments, share approximately 1/3 of their SNP polymorphisms, and so BXD and AXB/BXA mice would be expected to share only 1/9 of their genotypes. Therefore, it is not surprising that we found different QTLs for ventral and dorsal hippocampal volume in the BXD and AXB/BXA RI strain sets. Also, if C57BL/6, DBA/2 and AJ mice share the same genotype at other loci that influences hippocampal volume, that locus would not have been detected. Thus, the QTLs found in this study to influence dorsal and ventral hippocampal volume do not represent all possible QTLs, but only those segregating in these specific crosses.

There were also behavioral correlates of ventral and dorsal hippocampal volumes in the BXD strain set. BXD strains with smaller hippocampal volumes showed increased acoustic startle response and better performance on a test of spatial memory. The latter correlation was surprising, because in both rodents and in humans, spatial memory performance has been associated with the integrity of dorsal (posterior) hippocampus function (Bannerman *et al.* 2004; Moser &

Moser 1998). However, posterior hippocampal volume has not always been found to correlate with performance on tests of spatial memory in humans (Maguire *et al.* 2003). Interestingly, our finding of smaller hippocampal volume being correlated with better performance is in accord with the findings of a recent meta-analysis, in which smaller hippocampal volumes were associated with better memory performance in children, adolescents and young adults (van Petten 2004).

Despite the correlation between hippocampal volume and spatial memory performance, there were no associations between the QTL peak markers and performance on tasks of memory. However, tasks of anxiety-related behaviors (vertical activity, exploratory behavior and light/dark test performance), which may be linked to ventral hippocampal function (Bannerman *et al.* 2004), were correlated with ventral and dorsal hippocampal volume QTLs. Thus, ventral and dorsal hippocampus-dependent behaviors did not segregate in terms of their association with ventral and dorsal hippocampal volume QTLs. However, this does not rule out the possibility that ventral and dorsal hippocampal volume predicts ventral and dorsal hippocampus-dependent behaviors, because we were examining the genetic correlation between hippocampal volume and function across strains. Our results may have been different had we examined volume-function relationships across individual animals. The presence of a correlation between hippocampal volume and function across individual animals but not strains would suggest that environment or experience mediates the relationship. Importantly, these results were obtained by comparing a limited number of strains and volume, and behavioral data were not collected in all of the same animals. Therefore, these results are preliminary and will need to be replicated in larger samples, and other strain sets before inferences can be made about their relevance to structure-function relationships in humans.

The results of this study may have important implications for current efforts to understand genetic influences on neuropsychiatric disorders, such as schizophrenia. Schizophrenics and their non-psychotic siblings have been reported to have localized loss of anterior hippocampal volume (Csernansky *et al.* 2002; Tepest *et al.* 2003; for meta-analysis see Nelson *et al.* 1998). Therefore, genes that influence ventral or anterior hippocampal volume could represent susceptibility genes for schizophrenia. These findings may also have implications for depression and temporal lobe epilepsy, which have also been associated with reductions in the volume of the hippocampus. Major depression has been associated with a disproportionate decrease in posterior hippocampal volume as compared with controls (Neumeister *et al.* 2005). In temporal lobe epilepsy, there are mixed reports, but findings suggest a disproportionate reduction in the anterior hippocampal volume (Bernasconi *et al.* 2003). However, it is unknown whether these volume reductions are influenced by the presence of the disease

state or developmental influences on hippocampus growth prior to disease onset.

In summary, this study is the first to show independent genetic influences on ventral vs. dorsal hippocampal volume. Independent genetic influences were found in two lines of RI mice, showing that the independence of genetic influences on ventral and dorsal hippocampal volume can be generalized. Determination of the specific genes which exert these influences on ventral and dorsal hippocampal volume remains to be accomplished but could be useful for understanding the development of these functionally distinct sub-regions of the hippocampus complex and for identifying novel candidate genes for schizophrenia and other neuropsychiatric disorders in which the abnormal development of the hippocampus is thought to play a role.

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Acknowledgments

This study was supported by MH071616, NIMH, NIDA and NIAAA (2P20-DA21131), MouseBIRN (R24 RR021760), and NIAAA INIA (U01AA13499, U24AA13513).