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Q4 Variable impact of chronic stress on spatial learning and memory in 2 BXD mice

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10 HIGHLIGHTS

11

- 12 • Genetic loci identified that predict spatial memory performance
- 13 • The effect of stress on spatial memory performance was unique to genetic strain.
- 14 • Stress exposure reveals unique genetic predictors for spatial memory performance.
- 15 • Spatial learning ability is a consequence of genes and chronic stress history.

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50 1. Introduction

51 Stress has a complex relationship with learning and cognitive per-
52 formance, and stress can create both positive and negative effects de-
53 pending on duration, stressor, and environment. While many studies
54 have focused on acute stress, a period of chronic stress derived from
55 varying psychological and/or physiological stressors in an unpredictable
56 pattern can also impact cognitive performance [12,21,42]. Furthermore,
57 the response to chronic stress in both humans and rodents demon-
58 strates tremendous variability in performance. Studies vary as to the

ABSTRACT

The effects of chronic stress on learning are highly variable across individuals. This variability stems from gene–environment interactions. However, the mechanisms by which stress affects genetic predictors of learning are unclear. Thus, we aim to determine whether the genetic pathways that predict spatial memory performance are altered by previous exposure to chronic stress. Sixty-two BXD recombinant inbred strains of mice, as well as parent strains C57BL/6J and DBA/2J, were randomly assigned as behavioral control or to a chronic variable stress paradigm and then underwent behavioral testing to assess spatial memory and learning performance using the Morris water maze. Quantitative trait loci (QTL) mapping was completed for average escape latency times for both control and stress animals. Loci on chromosomes 5 and 10 were found in both control and stress environmental populations; eight additional loci were found to be unique to either the control or stress environment. In sum, results indicate that certain genetic loci predict spatial memory performance regardless of prior stress exposure, while exposure to stress also reveals unique genetic predictors of training during the memory task. Thus, we find that genetic predictors contributing to spatial learning and memory are susceptible to the presence of chronic stress.

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59 effects of chronic unpredictable stress on spatial learning and memory
60 as characterized by the Morris water maze; some studies have demon-
61 strated increased latency to platform times [21,42], while others have
62 shown decreased times due to a change in search strategies [12]. Addi-
63 tionally, chronic variable stress modulates hippocampal long-term po-
64 tentiation, a mechanism that is associated with performance on water
65 maze performance in rodents [6,8,25,35].

66 Response greatly varies across individuals as well, indicating a
67 strong genetic component. Gene–environment interactions have been
68 identified for the presence of stress and the development of Post-
69 Traumatic Stress Disorder [10,18] and fear learning [3]. However, the in-
70 teractions of genes and chronic stress on spatial learning and memory
71 have yet to be elucidated. To study the unique interactions between
72 gene and environment on cognitive performance, we have used the

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behavioral genetics model of BXD recombinant inbred mice, derived from C57BL/6J and DBA/2J parent strains [25,30,39]. In this model, individual differences in behavioral phenotype are correlated to variations in stretches of DNA through quantitative trait loci (QTL) analysis [7,9,25,39]. These chromosomal regions of DNA are then further analyzed to identify genes contributing to the phenotypic trait analyzed.

In this study, we assess spatial learning and memory in 62 BXD strains and C57BL/6J and DBA/2J parental strains via Morris water maze (MWM) performance. QTL mapping identified unique genetic loci and candidate genes associated with spatial learning performance in control and chronic stress conditions. Our results indicate that a chronic stress environment alters the genetic predictors of spatial learning, revealing an environment–gene interaction.

2. Materials and methods

2.1. Subjects

A total of 610 mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Parent strains C57BL/6J and DBA/2J ($n = 9$ –10 mice each) and 62 BXD strains ($n = 6$ –10 of each strain) were subjected to 4 weeks of behavioral testing starting at age 9 weeks. Mice were singly-housed, provided standard chow and water ad libitum, and kept on a 12 h light/dark cycle. Room temperature and humidity were maintained between 18–24 °C and 30–70%, respectively, with average temperature and humidity remaining at 21 °C and 35%. All procedures were approved by the Wright–Patterson Air Force Base (WPAFB) Institutional Animal Care and Use Committee and performed in accordance with the National Institute of Health standards and the Guide for the Care and Use of Laboratory Animals [1].

2.2. Experimental design

Ten cohorts of 50–66 animals were utilized. Strains utilized within each cohort were assigned randomly. Two littermates per strain were tested in a cohort: one was designated behavioral control (BC), and the other placed through a chronic variable stress (CVS) paradigm. Control animals ($n = 305$) underwent behavioral testing for three weeks, while CVS animals ($n = 305$) were placed through the CVS paradigm for one week prior to starting the three week behavioral testing period (elevated plus maze, MWM, and fear conditioning) (Fig. 1).

Analysis of emotional behavior during elevated plus maze and fear conditioning is reported in Carhuatanta, 2014. Animals completed an elevated plus maze test the day prior to starting the MWM test. The CVS stressors continued throughout behavioral testing. Cages were changed and body weights measured on a weekly basis. The entirety of testing was conducted over the course of 13 months.

2.3. Chronic variable stress

Animals in the Stress population underwent CVS treatment for four consecutive weeks, starting one week prior to the three week behavioral testing period. Five stressors were presented in random order with one stressor presented in the morning (0700–1100) and one in the afternoon (1300–1700), with the exception of novel overnight housing, which occurred overnight. The stressors were as follows: novel overnight housing, in which the mouse was singly housed in a

novel rat cage with ad libitum access to food and water; hypoxia, in which for 30 min the mice were placed in a low oxygen environment (8–12% oxygen); open field, in which the mice were singly housed in an open cage (10.5" × 19" × 8") in a well-lit area for 30 min; cold room, in which the mice were placed at 4 °C for 15 min in a cage devoid of bedding, singly housed; and constant motion, in which the mice were placed on an orbital shaker at 100 rpm for 1 h. Each stressor was repeated five times throughout the experiment with the exception of novel overnight housing, which was repeated three times. The combination and sequence of stressors changed each week to prevent predictability and limit habituation.

2.4. Morris water maze

The MWM tests the spatial navigation and memory of the mouse, as measured through the latency to find the hidden platform [2,6,26,35]. MWM training was conducted using a 90 cm diameter round basin filled to a water depth of 42 cm. Water was mixed with small quantities of nontoxic white tempera paint until opaque. Temperature was maintained at 19.5–24 °C, with an average temperature of 21 °C. A clear platform (6 cm diameter) was located approximately 0.5 cm below the water in the southwest quadrant. Five training days were completed, each with four 60 s trials with a randomized starting position (North, South, East, or West). On the first training day, if the animal did not reach the platform within 40 s a visible cue was placed on the platform. Five days of training was followed by a 30 s probe trial (24 h post last training session), in which the platform was removed. Immediately following probe trials, mice began a 2 day reversal period, in which the platform was moved from the southwest quadrant to the northeast quadrant (4 trials/day). Mouse swim path, position, speed, and latency to platform were recorded using EthoVision XT 7.0.418 cameras and software (Noldus Information Technology, Wageningen, The Netherlands). Here we assess latency to platform averaged across all trials, training trials, and reversal trials as a measure of learning and memory. Additionally, number of entries into the platform region of the maze during probe was assessed as an index of memory.

2.5. Statistical analysis

Performance was assessed as average latency to platform for all trials, training trials, and reversal trials as well as the number of entries into the platform area during probe. Stress-effect was calculated as the difference in performance between control and CVS littermates (CVS minus control). Mixed model analysis was then performed using lme4 and lme Test packages in R (fixed variables: stress, strain, and month of testing; random variable: cohort). A non-linear mixed effect test was performed followed by ANOVA to determine fixed variable main effects. Pearson product-moment correlations (R) and Spearman rank order correlations (ρ) across latency to platform time intervals were computed using GeneNetwork for the Control population, Stress population, and Stress-Effect.

Heritability of latency to platform average time for control and CVS populations was calculated for both broad- and narrow-sense variability following the Hegmann and Possidente method [14]. Narrow-sense heritability is defined as $h^2 = \frac{1}{2}V_A / (\frac{1}{2}V_A + V_W)$, where V_A = variance among strains and V_W = variance within strains. Variance within strains represents the environmental component of the variance, while

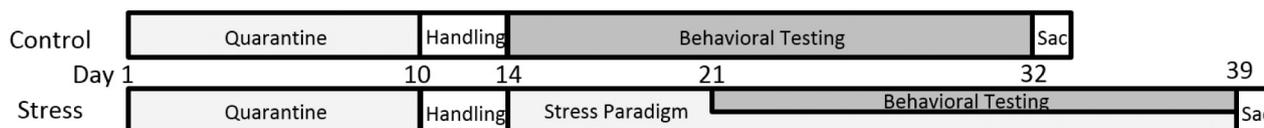


Fig. 1. Experimental design. Control animals underwent three weeks of behavioral testing starting on day 14, while animals designated as Chronic Variable Stress first underwent one week of the stress paradigm and began behavioral testing on day 21.

176 variance among strains represents the genetic factors [29]. Broad-sense
177 heritability, H^2 , was defined as V_A / V_T , where V_T = total population
178 variance.

179 The GeneNetwork suite of web tools (www.genenetwork.org) was
180 used for QTL mapping. GeneNetwork links differences in phenotype to
181 genomic regions using 89 BXD recombinant inbred strains that have
182 been assessed using 3806 genomic markers to identify suggestive and
183 significant QTLs with likelihood ratio statistics with genome-wide prob-
184 abilities of 0.67 and 0.05, respectively (GeneNetwork.org, [41]). QTLs
185 identified in this study are described by their greatest LRS value, signifi-
186 cance threshold passed, and confidence interval (determined via the
187 1LOD drop method [17]).

188 Candidate genes were identified as genes within the confidence in-
189 terval of each QTL that have a human homologue and/or have cis-
190 expression. Cis-expression in various brain tissues (amygdala, brain,
191 cerebellum, hippocampus, hypothalamus, midbrain, neocortex, nucleus
192 accumbens, and prefrontal cortex) was determined using the QTLminer
193 tool of GeneNetwork. Genes were further assessed via literary search
194 using PubMed for established relationships to stress, learning, memory,
195 and anxiety.

196 3. Results

197 3.1. MWM performance in BXD mice

198 An average of the latency to platform of all trials, training trials and
199 reversal trials was assessed for all 62 BXD strains and parental strains
200 (Fig. 2). Great variability was seen across strains in each time period,

201 resulting in 2.4 fold (all trials – Control) to 3.9 fold (reversal trials –
202 Stress) differences.

203 Averages of all mice in Control and Stress populations during each
204 trial are depicted in Fig. 3a. Both populations displayed a reduction in la-
205 tency to platform signifying learning. On average, mice subjected to CVS
206 stress displayed shorter LTP times throughout the task, however, the ef-
207 fect of stress varied greatly across strains.

208 Number of entries into the platform area during the probe trial (on
209 Day 6 prior to reversal trials) is shown in Fig. 3b. Average frequency of
210 entry per strain spanned from 0 to 2.25 entries for Control, and 0 to
211 3.4 for Stress. Of note, 58% and 47% of mice failed to enter platform re-
212 gion during the probe in Control and Stress populations, respectively.
213 Moreover, the Stress-effect on probe performance (Stress-Control, within
214 littermate) resulted in no difference in 65% of littermate pairs.

215 The latency to platform averages was tested for correlation in both
216 the control environment (Table 1) and stress (Table 2) environment. La-
217 tency to platform was strongly correlated across all time periods in both
218 control and stress conditions. Stress effect, the difference in latency to
219 platform (Stress-Control), for the latency to platform across all trials
220 and during training trials were significantly correlated, while a trend
221 was seen relating the all trials and reversal trials in both control and
222 stress conditions. **Q12**

223 3.2. Heritability of MWM performance

224 Broad-sense heritability and narrow-sense heritability were calcu-
225 lated to determine the proportion of variance across strains attributable
226 to genetic variance (Table 3). A strong genetic influence on a trait is

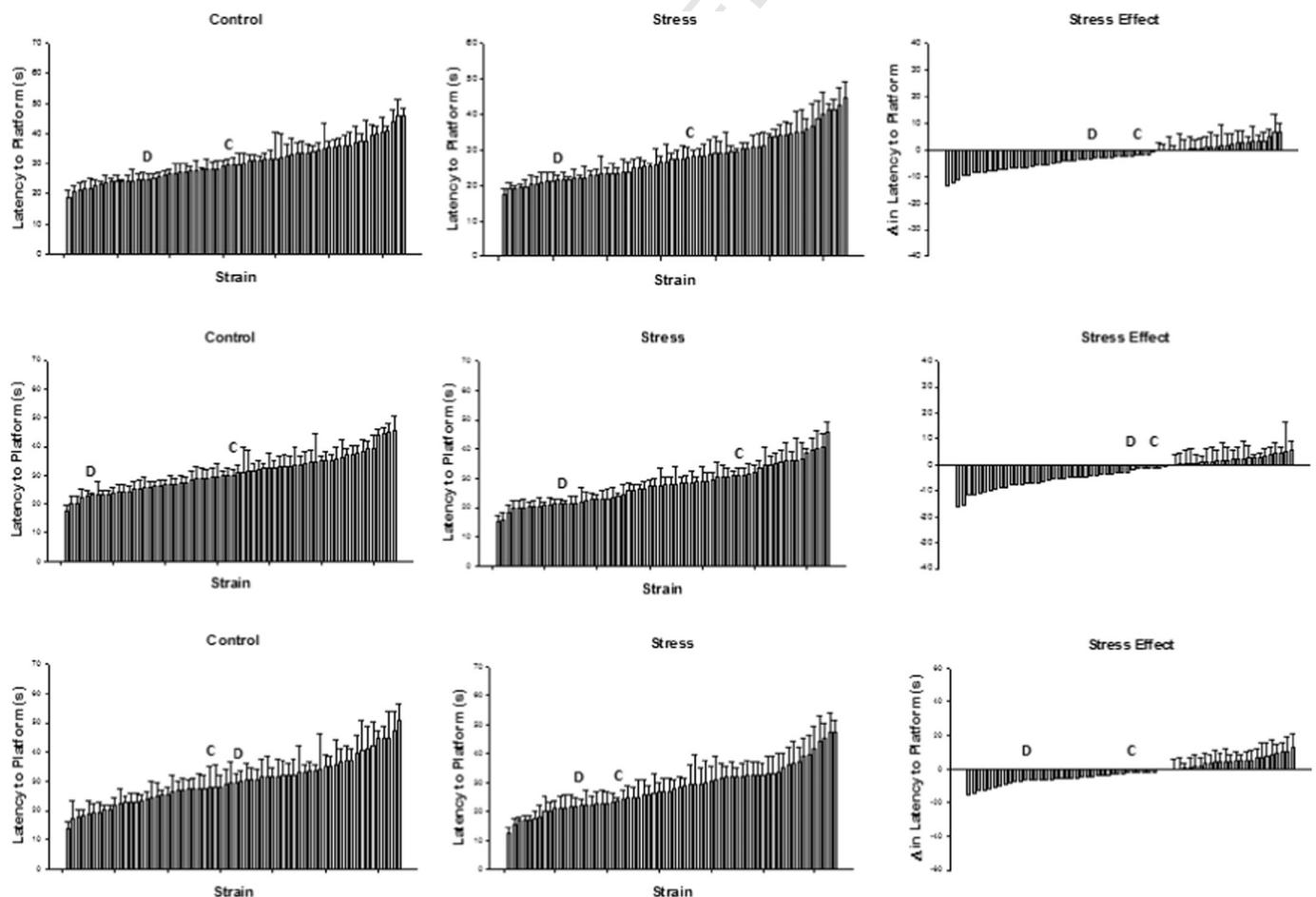


Fig. 2. Spatial learning performance of BXD strains. Mean \pm SEM latency to platform for Control (left), Stress (middle), and mean \pm SEM difference in latency to platform (Stress minus Control; Stress-Effect, right) of the average of A) all, B) training, and C) reversal trials during the MWM. Panels C and D indicate the locations of parental strains C57BL/6 and DBA/2 among the rankings, respectively.

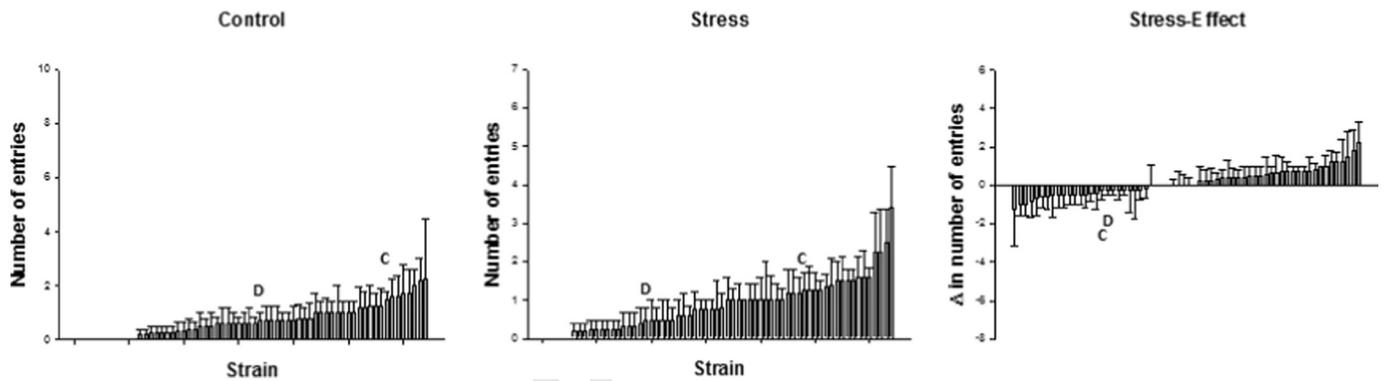
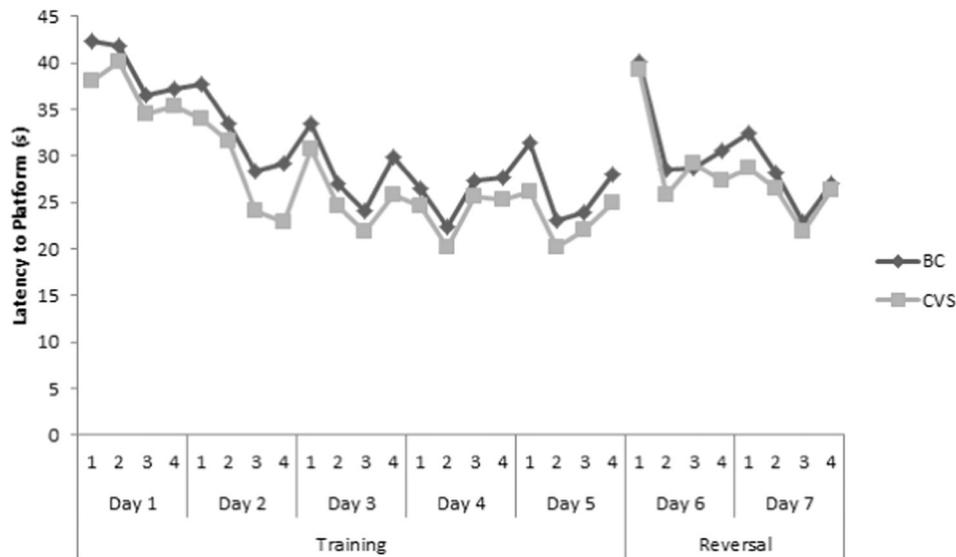


Fig. 3. A). Latency to platform during the MWM for the control and chronic stress populations. Mean \pm SEM latencies to platform were averaged across all animals for each trial (error bars too small to be seen). B). MWM probe performance. Mean \pm SEM number of entries into hidden platform region for Control (left), Stress (middle), and mean \pm SEM difference in number of entries (Stress minus Control; Stress-Effect, right) during probe test of MWM. Panels C and D indicate the locations of parental strains C57BL/6 and DBA/2 among the rankings, respectively.

Table 1
Correlation summary of LTP averages in Control population.

		Spearman rank correlation (ρ)							
		All LTP	SE All LTP	Tr LTP	SE Tr LTP	Rev LTP	SE Rev. LTP	Probe f	SE Probe f
Pearson r	All LTP	n = 64	-0.315	0.958*	-0.314	0.877*	-0.213	-0.515	-0.042
	SE All LTP	n = 64	-0.307	-0.265	0.904*	-0.307	0.627	0.11	-0.115
	Tr LTP	n = 64	0.968*	-0.276	-0.334	0.722*	-0.059	-0.502	-0.025
	SE Tr LTP	n = 64	-0.295	0.909*	-0.334	-0.164	0.278	0.131	-0.139
	Rev LTP	n = 64	0.867*	-0.308	0.713*	-0.16	-0.451	-0.417	-0.083
	SE Rev. LTP	n = 64	-0.171	0.65	-0.025	0.286	-0.427	0.101	-0.008
	Probe f	n = 64	-0.447	0.088	-0.435	0.11	-0.385	0.029	-0.505
	SE Probe f	n = 64	-0.081	-0.143	-0.087	-0.177	-0.053	-0.016	-0.467

determined by $h^2 \geq 0.25$. Strong heritability was seen in the average of the overall and training periods of both Control and Stress populations. Of note, the stress effect of each performance trait was found to have weak heritability ($h^2 = 0.09-0.12$). Traits that did not meet the requirement for strong heritability ($h^2 \geq 0.25$) were not assessed further.

3.3. Main effects of Stress, Strain, and Month of testing on latency to platform in the MWM

A non-linear mixed-effect test followed by ANOVA was run to determine the effects of Strain, Stress, and Month of testing for each trait measured (Table 4). Effects of stress and strain were seen for latency

Table 2
Correlation summary of LTP averages in Stress population.

		Spearman Rank Correlation (rho)							
		All LTP	SE All LTP	Tr LTP	SE Tr LTP	Rev LTP	SE Rev. LTP	Probe f	SE Probe f
Q2	All LTP	n = 64	0.415	0.959*	0.358	0.825*	0.247	-0.51	-0.118
t2.6	SE All LTP	0.388	n = 64	0.472	0.904*	0.221	0.627	-0.072	-0.115
t2.7	Tr LTP	0.971*	0.431	n = 64	0.47	0.655	0.174	-0.478	-0.141
t2.8	SE Tr LTP	0.337	0.909*	0.438	n = 64	0.074	0.278	-0.071	-0.139
t2.9	Rev LTP	0.865*	0.22	0.72	0.058	n = 64	0.345	-0.428	-0.022
t2.10	SE Rev. LTP	0.28	0.65	0.2	0.286	0.395	n = 64	0.027	-0.008
t2.11	Probe f	-0.524	-0.071	-0.53	-0.092	-0.407	0.008	n = 64	0.524
t2.12	SE Probe f	-0.177	-0.143	-0.213	-0.177	-0.061	-0.016	0.631	n = 64

to platform across all trials and during training, supporting the use of QTL mapping using this population and indicating that the unique genetic background of each strain contributed to the averages in each time period. Lastly, no effect for Month of testing was seen in averages of latency to platform of all trials and training trials, indicating that time of year (month) did not contribute significantly to variance observed across cohorts.

3.4. QTL mapping of MWM latency to platform

Significant QTLs were found for average latency to platform in all trials and training trials for the Stress population (Fig. 4). QTLs with suggestive LRS scores were found for average latency to platform across all trials and during training in both the Control and Stress populations (Fig. 4). A heatmap depicting the genetic mapping (Fig. 5) reveals the overlap of peaks identified to allow comparison across phenotypic traits and the presence of stress. QTLs unique to the Control population were located on Chromosomes 3, 7, and 10 for all trials and Chromosome 19 for all trials and during training. Peaks only found in the Stress population were identified on Chromosomes 1, 2, and 18 for all trials and training trials, and on Chromosome 8 for Training trials. Of interest, two peaks were found in both populations for average latency to platform across all trials and during training (on Chromosomes 5 and 10). Table 5 provides a summary of each of these peaks.

3.5. Candidate gene analysis

Genes within each QTL's confidence interval (determined by 1LOD drop method) were assessed for human homologues and cis-regulation to achieve a list of candidate genes (Table 6).

4. Discussion

We have assessed the spatial learning performance of 62 BXD strains and C57Bl/6J and DBA 2A parental strains in the presence and absence of a chronic stress environment. The purpose of this study was to determine whether the predictive relationship between genetic background and spatial memory performance would be unique to environmental context with exposure to chronic stress. Our results show that unique genetic maps were found in our Control and Stress populations. Of interest, novel QTLs associated with spatial learning in the chronic stress environment were identified (see Table 7).

Stress treatment elicited significant varied effects on latency to platform during the MWM (all trials and training trials). These effects can be seen in the varied average latencies to platform across each strain (Fig. 1), our mixed-model analysis findings identifying main effects due to chronic stress (Table 2), and, lastly, in the genetic loci identified associates with mouse spatial memory performance unique to Control and Stress populations (Figs. 3 and 4). Although latency to platform was the primary measure of spatial learning in this study it is possible that further investigations on other MWM measures, such as swim path, could provide addition information. Several of the QTLs reported here, especially those found in the Stress population, have not been found previously. Of note, no QTLs achieved significant LRS scores in the Control population; however, some did correspond with significant QTLs in the Stress population. Here we discuss QTLs that reached a significant LRS level.

4.1. QTLs and candidate genes for spatial learning within a chronic stress environment

Significant peaks unique for chronic stress on spatial learning performance were found on Chromosomes 1, 2, and 18 (Fig. 3). QTL 18 is a novel QTL for spatial performance that was unique to the Stress population for average latency to platform during all and training trials. This genetic region contains *Cdh2* and *Dsc1*, each contributing to cadherin's role synapse formation and maintaining neuronal circuits ([32]; Zhang, 2013; [43]). Additionally, the cis-regulated candidate gene, *Ttr* (transthyretin) is found within this region. This gene is of particular interest for our studies, because the *Ttr* protein has been associated with both protective and degenerative effects on brain function. In response to physiological insult, including aging and chronic stress, *Ttr* is expressed and circulates in the cerebrospinal fluid to act as a scavenger of Aβ peptide [20,23]. Nonetheless, mutant forms of *Ttr* are associated with Alzheimer's disease. Lastly, knockout of *Ttr* results in decreased spatial learning in the MWM that is not subject to further decrement with aging [34]. The relationship of *Ttr* with performance and its change in expression due to stress suggest a strong linkage of this gene as a contributor to our data set.

QTL 1 was significant for latency to platform in both all and training trials. This region has been previously identified for swimming speed and latency to platform over various trials [15,25]. Correlation of our averages across all and training trials to Milhau's resulted in a significant correlation (Spearman's rho = 0.82, P < 0.001) (2002); GeneNetwork). Of interest, this QTL resides within what has been described as the emotionality locus of Chromosome 1 [38]. Several candidate genes within this locus have established relationships with stress and anxiety

Table 3
Heritability of MWM performance.

	Control H ² /h ²	Stress H ² /h ²	SE H ² /h ²
All	0.52/0.28	0.53/0.30	0.24/0.11
Training	0.49/0.26	0.51/0.29	0.26/0.12
Reversal	0.41/0.20	0.42/0.21	0.19/0.09
Probe (f)	0.26/0.12	0.35/0.18	0.23/0.10

Table 4
Summary of One-way ANOVA results following linear mixed model fit.

	Strain P, F, df	Stress P, F, df	Month P, F, df
All	<0.0001, 7.14, 63	<0.0001, 20.86, 1	1, 0.01, 9
Training	<0.0001, 6.27, 63	<0.0001, 24.51, 1	1, 0.02, 9

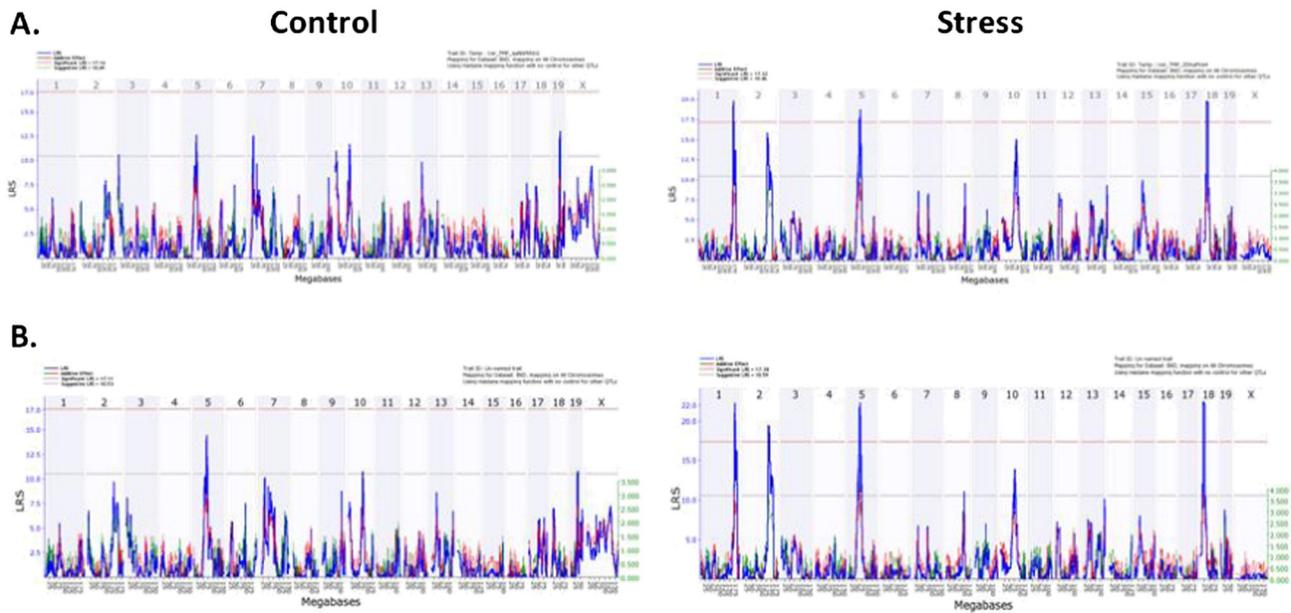


Fig. 4. Individual QTL maps of spatial learning. QTL maps of latency to platform during A) all and B) training trials for the Control (right) and Chronic Stress (left) populations.

316 (*Fcer1g*, *Ppox*, *Cadm3*, *Atp1a2*). *Fcer1g* encodes for a high affinity IgG re-
 317 ceptor. Expression of *Fcer1g* and *Ppox* is modulated by chronic mild
 318 stress [20] and footshock stress, respectively [4,5]. Knockout of the cell
 319 adhesion molecule, *Cadm3* results in an increase in anxiety and aggres-
 320 sion [37]. Genetic manipulation of *Atp1a2*, a P-type ATPase, results in in-
 321 creased fear, anxiety, and impaired learning [13]. A downregulation of
 322 *Atp1a2* in heterozygous animals impairs spatial learning and locomotor

activity, while increasing anxiety [27]. Mutations of this gene found in 323
 humans result in migraines and impaired nonverbal learning [31]. 324
 Taken together, these results suggest that *Fcer1g*, *Ppox* and *Cadm3* may 325
 be markers for the presence of stress, while *Atp1a2* may mark spatial 326
 learning performance within a stressful context. 327

A novel QTL found on chromosome 2 was identified in the Stress pop- 328
 ulation. A region nearby (130.8 Mb) to our locus (131.6–139.2 Mb) has 329

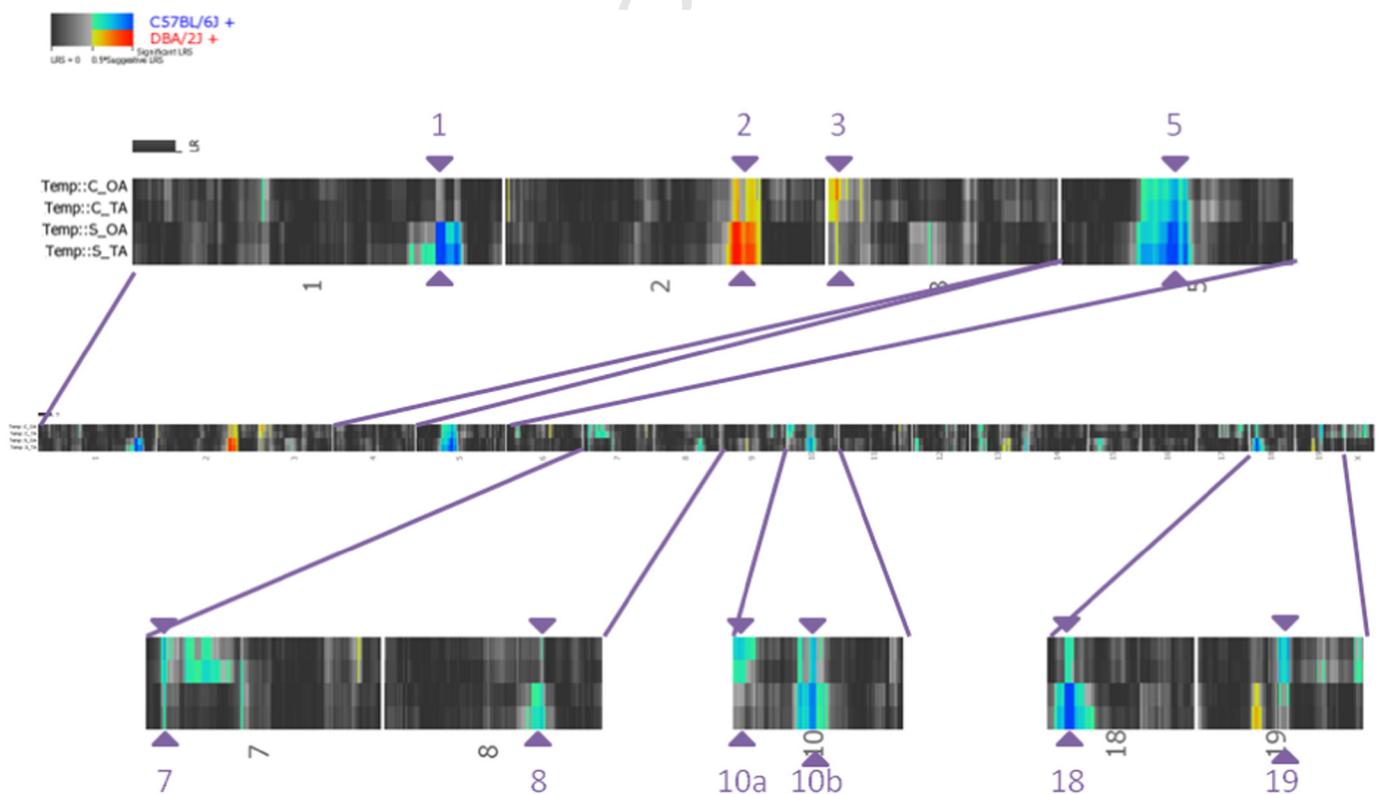


Fig. 5. Heatmap of QTL mapping of latency to platform during all and training trials containing significant and/or suggestive peaks. Dark red represents significant likelihood ratio score associated with DBA/2J genotype. Dark blue represents significant likelihood ratio score of trait associated with C57BL/6J genotype. Abbreviations in legend: All trials (A), Training trials (T), control (C), and chronic stress (S). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5
QTLs identified for latency to platform.

t5.3	QTL	Chromosome	Trait	Group	Location	Peak LRS	Peak marker	
t5.4	1	1	All	CVS	171.9–175.4	18.787	NES13029525	
t5.5	1	1	Training	CVS	171.7–175.5	22.301	NES13029525	
t5.6	2	2	All	CVS	131.6–139.6	16.054	CEL-2_135876979	
t5.7	2	2	Training	CVS	131.6–139.2	18.463	CEL-2_135876979	
t5.8	3	3	All	BC	7.7–10.1	11.646	rs3668064	
t5.9	5	5	All	BC	63.3–66.8	12.589	rs3656989	
t5.10	5	5	Training	BC	63.4–69.5	14.415	rs3656989	
t5.11	5	5	All	CVS	60.6–66.8	17.743	rs3657916	
t5.12	5	5	Training	CVS	60.6–68.0	22.376	rs3657916	
t5.13	7	7	All	BC	28.2–31.2	11.841	rs4226520	
t5.14	8	8	Training	CVS	93.1–98.7	11.12	rs3666069	
t5.15	10a	10	All	BC	0–14.7	10.599	D10Mit28	
t5.16	10b	10	All	BC	73.0–78.8	11.158	rs13480657	
t5.17	10b	10	Training	BC	73.2–79.8	10.711	rs13480653	
t5.18	10b	10	All	CVS	66.7–67.8,	66.7–67.8,	14.21	rs13480650
t5.19	10b	10	Training	CVS	72.3–78.5	13.901	rs13480653	
t5.20	18	18	All	CVS	16.1–24.2	19.218	rs6358426	
t5.21	18	18	Training	CVS	16.1–24.1	22.497	rs6358426	
t5.22	19	19	All	BC	33.5–38.1	12.373	rs3653886	

330 been identified previously for latency to platform [25] and location recog-
 331 nition crossovers in platform location during probe [40]. Our peak may
 332 overlap with these previous findings as fewer strains were used in
 333 Wehner [40], which can affect resolution of QTL mapping. Within QTL 2,
 334 genes for phospholipase C β (*Plcb1* and *Plcb4*) reside. Both *Plcb1* and
 335 *Plcb4* are associated with anxiety [22,33,16]. *Plcb1* is downregulated

Table 6
Gene list, QTLs for latency to platform times in control environment.

t6.1	Chr	Mapping location (Mb)	Gene List
t6.3	3	7.7–10.1	<i>Stmn2*</i> , <i>Hey1</i> , <i>Mrps28</i> , <i>Tpd52*</i> , <i>Zbtb10</i> , <i>Zfp704*</i> , <i>Pag1</i> , <i>Fabp5*</i>
t6.5	5	63.3–66.8	<i>0610040J01Rik*</i> , <i>Rell1*</i> , <i>Pgm1*</i> , <i>Tbc1d1*</i> , <i>Gm168*</i> , <i>Klf3*</i> , <i>C230096K16Rik*</i> , <i>Trl1*</i> , <i>Trl6*</i> , <i>9130005N14Rik*</i> , <i>Klh5*</i> , <i>Wdr19</i> , <i>Rfc1*</i> , <i>Rpl9*</i> , <i>Lias</i> , <i>Ugdh*</i> , <i>1110003E01Rik*</i> , <i>Ube2k*</i> , <i>C030017G13Rik*</i> , <i>N4bp2</i> , <i>Rhoh</i> , <i>Chrna9</i> , <i>Nsun7*</i> , <i>Appb2*</i> , <i>Uchl1*</i> , <i>Limch1*</i> , <i>Phox2b*</i> , <i>Tmem33*</i> , <i>Slc30a9*</i> , <i>C330024D21Rik*</i> , <i>Atp8a1*</i>
t6.6	7	28.2–31.2	<i>Blvrb</i> , <i>Sertad3</i> , <i>Sertad1</i> , <i>Prx</i> , <i>Hipk4*</i> , <i>Pld3*</i> , <i>Akt2*</i> , <i>Map3k10</i> , <i>Zfp59*</i> , <i>Psmc4</i> , <i>Fbl*</i> , <i>Dyrk1b</i> , <i>Dil3*</i> , <i>Timm50</i> , <i>Supt5h</i> , <i>Rps16*</i> , <i>Plekhh2</i> , <i>Zfp36</i> , <i>Gmfg</i> , <i>Lrjn1*</i> , <i>Il28a</i> , <i>Il28b</i> , <i>1190020J12Rik*</i> , <i>Pak4</i> , <i>Fbxo27*</i> , <i>Fbxo17</i> , <i>Mrps12</i> , <i>Sars2</i> , <i>Nfkbb</i> , <i>Sirt2</i> , <i>Zfp420*</i> , <i>Ech1</i> , <i>Lgals4</i> , <i>Lgals7*</i> , <i>Capn12</i> , <i>Actn4*</i> , <i>Map4k1</i> , <i>Ryr1</i> , <i>Rasgrp4*</i> , <i>Ggn</i> , <i>Psmd8</i> , <i>Kcnk6</i> , <i>Yif1b</i> , <i>Spint2</i> , <i>Ppp1r14a</i> , <i>Dpf1</i> , <i>2310022K01Rik*</i> , <i>Zfp84*</i> , <i>Zfp30*</i> , <i>Zfp790*</i> , <i>BC027344*</i> , <i>2900035I09Rik*</i> , <i>Zfp27*</i> , <i>Zfp74*</i> , <i>Zfp568*</i> , <i>Zfp14*</i> , <i>Zfp82*</i> , <i>Zfp566*</i> , <i>Zfp260*</i> , <i>Zfp382*</i> , <i>Zfp146*</i> , <i>EG330503*</i> , <i>Cox7a1*</i> , <i>Capns1*</i> , <i>Polr2i*</i> , <i>Thap8*</i> , <i>Clip3*</i> , <i>Alkbh6*</i> , <i>Al4289*</i> , <i>0610010E21Rik*</i> , <i>Lrjn3</i> , <i>Tyropb*</i>
t6.7	10a	0–18.8	<i>Cnksr3*</i> , <i>Oprm1*</i> , <i>Rgs17*</i> , <i>Mtrf11*</i> , <i>Fbxo5</i> , <i>Vip</i> , <i>Myct1</i> , <i>Syme1*</i> , <i>Esr1</i> , <i>Zbtb2</i> , <i>Akap12</i> , <i>Mthfd11*</i> , <i>lyd*</i> , <i>Ppp1r14c*</i> , <i>Lrp11*</i> , <i>Pcmt1*</i> , <i>Nup43*</i> , <i>Lats1</i> , <i>Katna1*</i> , <i>Ppil4*</i> , <i>Zc3h12d*</i> , <i>Map3k7ip2</i> , <i>Ust*</i> , <i>Sash1*</i> , <i>Sand5*</i> , <i>Stxbp5*</i> , <i>9130014G24Rik*</i> , <i>Rab32*</i> , <i>Grm1</i> , <i>Shprh*</i> , <i>Fbxo30</i> , <i>Epm2a</i> , <i>Utrn*</i> , <i>B230208H11Rik*</i> , <i>Stx11*</i> , <i>Sf3b5*</i> , <i>Plagl1*</i> , <i>Ltv1*</i> , <i>Phactr2*</i> , <i>Fuca2*</i> , <i>Pex3*</i> , <i>Adat2*</i> , <i>Aig1*</i> , <i>6430706H07Rik*</i> , <i>A230061C15Rik*</i> , <i>Hivep2*</i> , <i>Gpr126*</i> , <i>9030203C11Rik*</i> , <i>Vta1*</i> , <i>Nmbr*</i> , <i>Cited2</i> , <i>Heca*</i> , <i>Reps1</i> , <i>Cccdc28a*</i> , <i>Nhs11*</i> , <i>Hebp2*</i> , <i>D10Bwg1379c*</i> , <i>Perop*</i>
t6.8	10b	73.0–78.8	<i>Pcdh15</i> , <i>Rtdr1</i> , <i>Gnaz</i> , <i>Rab36</i> , <i>Bcr</i> , <i>Adora2a</i> , <i>Upb1</i> , <i>Snrpd3</i> , <i>Ggt1</i> , <i>Ggtla1</i> , <i>Susd2</i> , <i>Cabin1</i> , <i>Ddt</i> , <i>Gstt1</i> , <i>Gstt2*</i> , <i>Mif</i> , <i>Derl3</i> , <i>Smarcb1</i> , <i>Mmp11</i> , <i>Ndg2*</i> , <i>Gm867*</i> , <i>Vpreb3</i> , <i>S100b</i> , <i>Mcm3ap</i> , <i>Lss</i> , <i>Ftcd</i> , <i>Col6a2</i> , <i>Col6a1</i> , <i>Pcbp3</i> , <i>Slc19a1</i> , <i>Col18a1</i> , <i>Pofut2</i> , <i>Adarb1*</i> , <i>Itgb2</i> , <i>Pttg1ip</i> , <i>Sumo3</i> , <i>Ube2g2</i> , <i>Krtap12-1</i> , <i>Lrrc3</i> , <i>Trpm2</i> , <i>Pfkl</i> , <i>Aire</i> , <i>Dnmt3l</i> , <i>Tmem1</i> , <i>Agpat3</i> , <i>Cstb</i> , <i>Pdxk*</i> , <i>Ilvbl</i> , <i>Casp14</i> , <i>Slc1a6</i>
t6.9	19	33.5–38.1	<i>Lipf</i> , <i>Ankrd22</i> , <i>Acta2</i> , <i>Fas</i> , <i>Ch25h*</i> , <i>Lipa</i> , <i>Ifit3</i> , <i>Ifit1</i> , <i>Slc16a12</i> , <i>Pank1</i> , <i>Mphosph1</i> , <i>Htr7</i> , <i>Rpp30</i> , <i>Ankrd1</i> , <i>Pcgf5</i> , <i>Hectd2*</i> , <i>Ppp1r3c</i> , <i>Tnka2</i> , <i>Btaf1*</i> , <i>Cpeb3*</i> , <i>Ide</i> , <i>Kif11</i> , <i>Hhex*</i> , <i>Cyp26c1</i> , <i>Cyp26a1</i> , <i>Fer113</i>

Table 7
Gene list, QTLs for latency to platform times in high-stress environment.

t7.1	Chr	Mapping location (Mb)	Gene list	t7.2
t7.3	1	171.7–175.5	<i>Hsd17b7</i> , <i>Ddr2*</i> , <i>Uap1</i> , <i>Uhmk1</i> , <i>Olfnl2b</i> , <i>Atf6</i> , <i>Dusp12</i> , <i>Fcgr2b</i> , <i>Fcgr3*</i> , <i>1700009P17Rik*</i> , <i>Sdhc*</i> , <i>Mpz</i> , <i>Pcp41*</i> , <i>Nr1i3*</i> , <i>Tomm40l*</i> , <i>Apoa2*</i> , <i>Fcer1g*</i> , <i>Ndufs2*</i> , <i>Adamts4*</i> , <i>B4galt3*</i> , <i>Ppox*</i> , <i>Usp21</i> , <i>Ufc1*</i> , <i>Dedd*</i> , <i>Nit1*</i> , <i>Pfdn2*</i> , <i>Pvrl4*</i> , <i>Arhgap30*</i> , <i>Uspf1*</i> , <i>F11r*</i> , <i>B930036N10Rik*</i> , <i>Refbp2*</i> , <i>Itln1*</i> , <i>Cd244</i> , <i>Ly9</i> , <i>Slamf7*</i> , <i>Cd48</i> , <i>Slamf1</i> , <i>Cd84*</i> , <i>Slamf6*</i> , <i>Vangl2*</i> , <i>Nhlh1</i> , <i>Ncstn*</i> , <i>Copa*</i> , <i>Pex19*</i> , <i>Atp1a4*</i> , <i>Wdr42a*</i> , <i>Pea15a</i> , <i>Casq1*</i> , <i>Atp1a2*</i> , <i>Igsf8*</i> , <i>Kcnj9*</i> , <i>Kcnj10*</i> , <i>Pigm*</i> , <i>Slamf9*</i> , <i>Igsf9*</i> , <i>Tagln2</i> , <i>Ccdc19*</i> , <i>Vsig8*</i> , <i>Slamf8*</i> , <i>Fcrl6*</i> , <i>Dusp23*</i> , <i>Crp</i> , <i>Apcs</i> , <i>Fcer1a</i> , <i>Darc*</i> , <i>Cadm3*</i> , <i>Aim2*</i>	t7.4
t7.5	2	131.6–139.2	<i>Prnp</i> , <i>Prnd</i> , <i>Rassf2*</i> , <i>Slc23a2</i> , <i>Pcna</i> , <i>Cds2</i> , <i>Chgb</i> , <i>Mcm8</i> , <i>2900022B07Rik*</i> , <i>Bmp2</i> , <i>Hao1</i> , <i>Plcb1*</i> , <i>Plcb4*</i> , <i>Pak7*</i> , <i>BC034902*</i> , <i>Ankrd35</i> , <i>Snapp25</i> , <i>Mkks</i> , <i>Jag1</i> , <i>Btdb3</i>	t7.6
t7.7	5	60.6–68.0	<i>Centd1</i> , <i>0610040J01Rik*</i> , <i>Rell1*</i> , <i>Pgm1*</i> , <i>Tbc1d1*</i> , <i>Gm1683*</i> , <i>Klf3*</i> , <i>C230096K16Rik*</i> , <i>Trl1*</i> , <i>Trl6*</i> , <i>9130005N14Rik*</i> , <i>Klh5*</i> , <i>Wdr19</i> , <i>Rfc1*</i> , <i>Rpl9*</i> , <i>Lias</i> , <i>Ugdh*</i> , <i>1110003E01Rik*</i> , <i>Ube2k*</i> , <i>N4bp2</i> , <i>Rhoh</i> , <i>Chrna9</i> , <i>Nsun7*</i> , <i>Appb2*</i> , <i>Uchl1*</i> , <i>Limch1*</i> , <i>Phox2b*</i> , <i>Tmem33*</i> , <i>Slc30a9*</i> , <i>Atp8a1*</i>	t7.8
t7.9	8	93.1–98.7	<i>Chd9*</i> , <i>Rb12*</i> , <i>Aktip*</i> , <i>Rprgrip1*</i> , <i>Fto*</i> , <i>Irx3*</i> , <i>D230002A01Rik*</i> , <i>4933436C20Rik*</i> , <i>Irx5*</i> , <i>Irx6</i> , <i>Mmp2*</i> , <i>Lpcat2*</i> , <i>Capns2*</i> , <i>Slc6a2</i> , <i>Ces1</i> , <i>Gnao1</i> , <i>Amfr</i> , <i>Bbs2</i> , <i>Mt4</i> , <i>Mt3</i> , <i>Nup93</i> , <i>Slc12a3</i> , <i>Herpud1</i> , <i>Nlrc5*</i> , <i>Tmem28</i> , <i>Al451557*</i> , <i>Cpne2*</i> , <i>Arl2bp</i> , <i>Cc122</i> , <i>Cx3cl1*</i> , <i>1700121C10Rik*</i> , <i>Ccl17*</i> , <i>Ciapin1*</i> , <i>Coq9*</i> , <i>Polr2c</i> , <i>Dok4*</i> , <i>Gpr114*</i> , <i>Gpr56*</i> , <i>Gpr97*</i> , <i>Katnb1*</i> , <i>Kifc3*</i> , <i>Cngb1</i> , <i>Zfp319*</i> , <i>AA960436*</i> , <i>Mmp15*</i> , <i>Gtl3</i> , <i>Csnk2a2*</i> , <i>4933406B17Rik*</i> , <i>Gins3*</i> , <i>Ndrge4*</i> , <i>Cnot1</i> , <i>Slc38a7*</i> , <i>Got2</i>	t7.10
t7.11	10	66.7–67.8; 68.8–78.9	<i>Nrbf2*</i> , <i>Egr2</i> , <i>Zfp365*</i> , <i>Rtkn2*</i> , <i>Arid5b*</i> , <i>Ank3*</i> , <i>Ccdc6</i> , <i>Slc16a9</i> , <i>Phyhlpl</i> , <i>Bicc1</i> , <i>Tfam</i> , <i>Ube2d1</i> , <i>Ipmk</i> , <i>Zwint*</i> , <i>Pcdh15</i> , <i>Rtdr1</i> , <i>Gnaz</i> , <i>Rab36</i> , <i>Bcr</i> , <i>Adora2a</i> , <i>Upb1</i> , <i>Snrpd3</i> , <i>Ggt1</i> , <i>Ggtla1</i> , <i>Susd2</i> , <i>Cabin1</i> , <i>Ddt</i> , <i>Gstt1</i> , <i>Gstt2*</i> , <i>Mif</i> , <i>Derl3</i> , <i>Smarcb1</i> , <i>Mmp11</i> , <i>Ndg2*</i> , <i>Gm867*</i> , <i>Vpreb3</i> , <i>S100b</i> , <i>Mcm3ap</i> , <i>Lss</i> , <i>Ftcd</i> , <i>Col6a2</i> , <i>Col6a1</i> , <i>Pcbp3</i> , <i>Slc19a1</i> , <i>Col18a1</i> , <i>Pofut2</i> , <i>Adarb1*</i> , <i>Itgb2</i> , <i>Pttg1ip</i> , <i>Sumo3</i> , <i>Ube2g2</i> , <i>Krtap12-1</i> , <i>Lrrc3</i> , <i>Trpm2</i> , <i>Pfkl</i> , <i>Aire</i> , <i>Dnmt3l</i> , <i>Tmem1</i> , <i>Agpat3</i> , <i>Cstb</i> , <i>Pdxk*</i> , <i>Ilvbl</i> , <i>Casp14</i> , <i>Slc1a6</i>	t7.12
t7.13	18	16.1–24.1	<i>Cdh2*</i> , <i>Dsc3</i> , <i>Dsc2</i> , <i>Dsc1</i> , <i>Dsg1b*</i> , <i>Dsg4</i> , <i>Dsg3</i> , <i>Dsg2*</i> , <i>Ttr</i> , <i>B4galt6</i> , <i>Rnf125</i> , <i>Rnf138*</i> , <i>Mep1b</i> , <i>Gm944*</i> , <i>Klh114</i> , <i>4921517011Rik*</i> , <i>Asxl3*</i> , <i>Nol4*</i> , <i>Dtna*</i> , <i>Mapre2*</i> , <i>Znf24</i>	t7.14

Bolded genes are those from QTL regions which overlap between traits (ie. All, Training, Reversal).

337 following chronic mild stress and repeated stress ([28] 2005, single and
 338 repeated stress-induced modulation of phospholipase C catalytic activity
 339 and expression: role in LH behavior). Knockout of *Plcb1* is used as a
 340 model for schizophrenia and shows deficits in location recognition and
 341 memory, while object recognition remains intact, indicating a deficit in
 342 hippocampal dependent learning [16,22,24]. Our experiments confirm a
 343 relationship of phospholipase C β with hippocampal-dependent cognitive
 344 performance and stress.

4.2. QTLs and candidate genes for spatial learning in either control or chronic stress environment

345 QTLs appearing for spatial learning performance in both Control and
 346 Stress populations were found on Chromosomes 5 and 10 (Fig. 3). The
 347 QTL on Chromosome 5 achieved significant LRS scores in the chronic
 348 stress population while only the suggestive level in the Control popula-
 349 tion. Interestingly, genes associated with neuronal plasticity were iden-
 350 tified (*Pgm1*, *Uchl1*, and *Atp8a1*) [11,19,36], supporting our hypothesis
 351 that genes within this region would be responsible for spatial learning
 352 regardless of the environmental context. Of interest, *Uchl1* expression
 353 rescues contextual memory in β amyloid models of Alzheimer disease
 354 [11]. Additionally, a deficiency of *Atp8a1* results in alterations of activity
 355 and decreased performance on the MWM [19]. These results indicate
 356 that the locus of QTL 5 contains genes important for hippocampal de-
 357 pendent learning in either control or chronic stress environments.

5. Conclusions

In summary, our results identified genetic loci for spatial learning in control and chronic stress environments. Several of these loci are novel for spatial learning performance and should be studied in future experiments. Nonetheless, within these loci are candidate genes that likely contribute to spatial learning performance in their respective environmental contexts. Our results indicate that performance on spatial learning tasks is influenced by both genetic background and chronic stress history.

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Broad-sense (H^2)/Narrow-sense (h^2) heritability of latency to platform within Control and Chronic Stress (Stress) populations and Stress-Effect (SE, Stress minus Control). Broad-sense: V_a / V_t ; Narrow-sense: $1/2 V_a / (1/2 V_a + V_w)$; V_a = variance among strains, V_t = variance across total population V_w = variance within strains.

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