Genetic architecture and behavioral analysis of attention and impulsivity

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Chapter 6

Insights in the genetic architecture of impulsivity in mice

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In preparation

Abstract

Impulsivity as observed in tasks of inhibitory response control is a heritable endophenotype of attention deficit hyperactivity disorder (ADHD). To identify genes underlying this variety of impulsivity we assessed inhibitory control of 43 BXD recombinant inbred strains in a five-choice serial reaction time task (5-CSRTT). QTL analysis indicated that impulsivity significantly mapped to a locus on chromosome 14. Candidate genes within QTL interval were identified by the presence of non-synonymous mutations (Ppyr1, Mmrn2, Wapal and 4930474N05Rik) and analysis of the genetic association of syntenic human genes with ADHD diagnosis (Nrg3). Impulsivity in the BXD strains did not correlate with response variability, which is a novel measure of lapses of attention and a well-replicated ADHD endophenotype. This was in line with the hypothesis that separate molecular pathways contribute to attention deficit and hyperactivity-impulsivity in this heterogeneous disorder. Future studies that identify the causative genes within the impulsivity QTL will help to understand the pathway contributing to symptoms of hyperactivity-impulsivity in ADHD.

Introduction

The multifaceted construct of impulsivity is associated with several psychiatric disorders, most notably attention deficit hyperactivity disorder (ADHD; DSM-IV, 1994). Numerous studies have indicated that symptomatic levels of impulsive behavior translate into deficits in tasks of inhibitory response control (Aron & Poldrack, 2005; Bidwell *et al.*, 2007), and that this relation is primarily genetic in origin (Young *et al.*, 2009b). Indeed, genetic factors influence inhibitory control, with heritability estimates in the range of 18 – 50% (Kuntsi *et al.*, 2006; Schachar *et al.*, 2010). As such, studies that aim to identify genetic variants underlying inhibitory control are instrumental in understanding parts of the etiology of ADHD. Moreover, since it is under debate whether symptoms of hyperactivity-impulsivity and inattention in ADHD reflect the same or separate disorders (Derefinko *et al.*, 2008; Diamond, 2005; Milich *et al.*, 2001), it is of interest to investigate whether genetic variants underlying inhibitory control affect attentional performance.

To identify quantitative genetic loci (QTLs) underlying impulsivity, we employed the panel of BXD recombinant inbred strains, which are derived from an intercross of C57BL/6J and DBA/2J (Peirce *et al.*, 2004). Previous studies have shown that these founder strains differ in impulsivity phenotypes, suggesting that the BXD recombinant inbred strains derived thereof are powerful resources to genetically dissect impulsivity (Helms *et al.*, 2006; Loos *et al.*, 2010b; Loos *et al.*, 2009; Pattij *et al.*, 2007; Young *et al.*, 2009a). Available databases of brain gene expression and single nucleotide polymorphisms (SNPs) of these strains aid to efficiently identify candidate genes within QTL regions (Chesler *et al.*, 2005).

In rodent research, several operant tasks of impulsivity exist (Evenden, 1999; Winstanley *et al.*, 2006a). Of the subset of tasks that assesses inhibitory control, the five-choice serial reaction time task (5-CSRTT) is the most widely used paradigm (Eagle & Baunez, 2010). In this task, premature responses to a food predictive stimulus are a measure of reduced response inhibition (Robbins, 2002). In line with human studies, inhibitory control in the 5-CSRTT is influenced by genetic variation, with an estimated heritability of 34% in a genetically diverse panel of inbred stains (Loos *et al.*, 2009).
The primary measure of attention in the 5-CSRTT is the accuracy of responding to a brief light stimulus in one of five response holes (response accuracy hereafter; Robbins, 2002). In addition, this task allows extracting intra-individual variability in correct response latencies (response variability hereafter), which is a novel measure of lapses of attention and one of the best-replicated ADHD endophenotypes (Castellanos & Tannock, 2002; Leth-Steensen *et al.*, 2000; Loos *et al.*, 2010b; Sabol *et al.*, 2003). Here, we investigated the relation between impulsivity (inhibitory control) and measures of attention in the same task. Correlation analyses across the recombinant inbred BXD strains (genetic correlation) with minimal influence of environmental factors (Crusio, 2006; Loos *et al.*, 2009) were used to evaluate the genetic relation between impulsivity and attention.

Materials and Methods

Animals

Parental and BXD lines were received from Jackson Lab, or from Oak Ridge Laboratory (BXD43, BXD51, BXD61, BXD62, BXD65, BXD68, BXD69, BXD73, BXD75, BXD87, BXD90), and were bred in the facility of the Neuro-Bsik consortium of the VU University Amsterdam. Seven-week-old male mice were singly housed on sawdust in standard Makrolon type II cages (26.5 cm long, 20.5 cm wide and 14.5 cm high) enriched with cardboard nesting material. After a habituation period of minimally 1 week, body weights were recorded and mice were subjected to one test of prepulse inhibition that lasted 40 min. In the subsequent week, mice were food-restricted to gradually decrease their body weight to 90 – 95% of their initial body weight before daily training in operant cages commenced (5 days each week). Water was available *ad libitum* throughout the experiment.

5-CSRTT

Mice were trained to perform the 5-CSRTT on an individually paced schedule, as described previously (Loos *et al.*, 2010b; Loos *et al.*, 2009). During the first week, mice underwent 1 habituation and 4 magazine training sessions. In the next week, mice were trained to perform an instrumental response into the stimulus

holes to earn a reward, and only commenced to 5-CSRTT training when they earned at least 50 rewards within one session. During 5-CSRTT training a trial started with a response of the subject into the illuminated magazine, which switched off magazine light and after an ITI of 5 s a stimulus in 1 of the 5 stimulus holes was presented for a limited duration (stimulus duration). A response in the correct stimulus hole within the limited hold time of 4 s after termination of the stimulus switched on the magazine light and delivered a food pellet. Both an incorrect response into a non-illuminated stimulus hole and an omission of a correct response resulted in a time-out period, during which all stimulus lights and the house light were turned off. When the time-out period ended, both the house light and the magazine light were switched on, and the subject could start the next trial. A premature response into a non-illuminated stimulus hole during the delay period also resulted in a time-out period, but a subsequent response into the illuminated magazine restarted the same trial. The percentage of omission errors was defined as [100 × (omissions) / (omissions + number of correct and incorrect responses)]. Response accuracy was defined as [100 × (number of correct responses) / (number of correct and incorrect responses)]. Impulsivity in terms of the percentage premature responses was defined as [100 × (number of premature responses) / (number of omissions + correct + incorrect responses)]. In the first 5-CSRTT session, the stimulus duration was set at 16 s, which was decreased in subsequent sessions to 8, 4, 2, 1.5 and 1 s as soon as the subject reached criterion performance (omissions < 30%, response accuracy > 60%, started trials > 50) or after 10 sessions. Intra-individual variability in correct response latencies (response variability in short) was defined by the standard deviation of the correct response latencies. The total number of sessions required to reach the stimulus duration of 1 s was used as measure of required training sessions. Dependent measures were calculated from the 6th until the 10th session at stimulus duration of 1 s, and the average of these sessions was used as standard 5-CSRTT performance. In the week following the 10th session, the ITI was programmed to vary (5, 7.5 and 12.5 s), with each interval occurring an equal number of times within session. Mice were excluded from analyses when they initiated fewer than 30 trials on average or made no correct or incorrect responses during two or more standard sessions. Strains that completed fewer than 50 trials on average in combination with magazine latencies greater than 4 s, together indicative of reduced motivation, were excluded.

Data analysis

For evaluation of strain differences and task manipulation, analysis of variance (ANOVA) or ANOVA with repeated measures were used, with paired Student's t-tests for post hoc testing. Estimates of the genetic effect size (narrow sense heritability) were calculated according to Hegmann and Possidente (Hegmann & Possidente, 1981) using a custom function (Microsoft Excel 2003) that takes the

differences in the number of animals per group into account when estimating the within- and between-strain variance (Lynch & Walsh, 1998) as previously implemented (Heimel *et al.*, 2008; Loos *et al.*, 2009). Interval mapping analysis was performed in GeneNetwork (www.genenetwork.org) that uses the embedded MapManager software (Manly *et al.*, 2001) to perform Haley–Knott regression. Empirical P-values, derived from 1000 permutations, were used to assess whether the peak of a QTL was statistically significant (P-value <0.05) or suggestive (on average one false positive per genome scan; genome-wide *P*-value <0.63; (Lander & Kruglyak, 1995). A one-LOD drop-off was used to determine the QTL confidence interval of each peak. GeneNetwork's gene expression database of prefrontal cortex tissue (Virginia Commonwealth University, BXD Prefrontal Cortex Saline Control M430 2.0 (Dec06) RMA Dataset, Accession number: GN135) was used to calculate Pearson correlations using GeneNetwork's online tools. For the analysis of non-synonymous mutations in genes under a QTL peak, GeneNetwork's comprehensive SNP browser was used (date: June 2010). NCBI's homologene datafiles (version July 13th 2009) were used to convert mouse gene coordinates (UCSC genome build 9) to syntenic human genome coordinates (NCBI human genome build 37.1), with an additional 2000 base pairs before and after each human gene. The online LiftOver tool in the USCS genome browser (http://genome.ucsc.edu/cgi-bin/hgLiftOver) was used to convert human genome coordinates from build 37 to build 36, to allow selection of SNPs in the syntenic human loci. Confirmation of gene-based association in human data was carried out using VEGAS software (version 0.8.27; Liu *et al.*, 2010). VEGAS applies a test that incorporates information from a set of markers within a gene (or region) and accounts for linkage disequilibrium (LD) between markers by using adaptive simulations from the multivariate normal distribution. An empirical P value of 0 from 100000 simulations can be interpreted as $P < 10^{-5}$, which exceeds a Bonferroni-corrected threshold at [0.05/(number of tested autosomal genes)]. The initial test for marker association with a trait was carried out using Plink software (Purcell *et al.*, 2007) using logistic regression or a transmission disequilibrium test on the family based sample. The results from Plink were used as input for the VEGAS gene-based test analysis. Human genetic datasets informative for ADHD was retrieved from the Database of Genotyped and Phenotypes (accession number phs000016.v2.p2). The ADHD data is from the International Multicentre ADHD genetics project (IMAGE), and consists of a family-based dataset of 913 ADHD cases and 1378 healthy controls. Standard quality control procedures were carried out as described previously (Lips *et al.*, 2011; Rizzi *et al.*, 2011).

Results

In total, we obtained 5-CSRTT data of 588 mice (**Supplementary Table 1**), distributed across 39 BXD strains (on average n = 13.1 per strain, minimum n = 6 per strain), the parental lines (C57BL/6J, n = 35; DBA/2J, n = 20) and reciprocal F1 lines (B6D2F1, n = 12; D2B6F1, n = 10). For technical reasons, 6 mice were not subjected to a variable ITI.

Impulsivity of BXD mice in the 5-CSRTT

Strains differed significantly in their level of impulsivity (**Fig. 1a**; percentage premature responses; $F(42, 545) = 5.00$, $P < 0.001$). The estimate of the genetic effect size for all 5-CSRTT parameters, as well as body weight-related parameters are shown in **Table 1**. Increasing the demand for inhibitory control by randomly increasing the ITI enhanced impulsivity (ITI: $F(1, 539) = 884.26$, $P < 0.001$; ITI × strain: $F(42,539) = 2.39$, $P < 0.001$) in all strains significantly (one-sample Student's t-test $P < 0.05$), except for BXD38 (**Fig. 1b**), indicating that all strains used a similar strategy to solve the task.

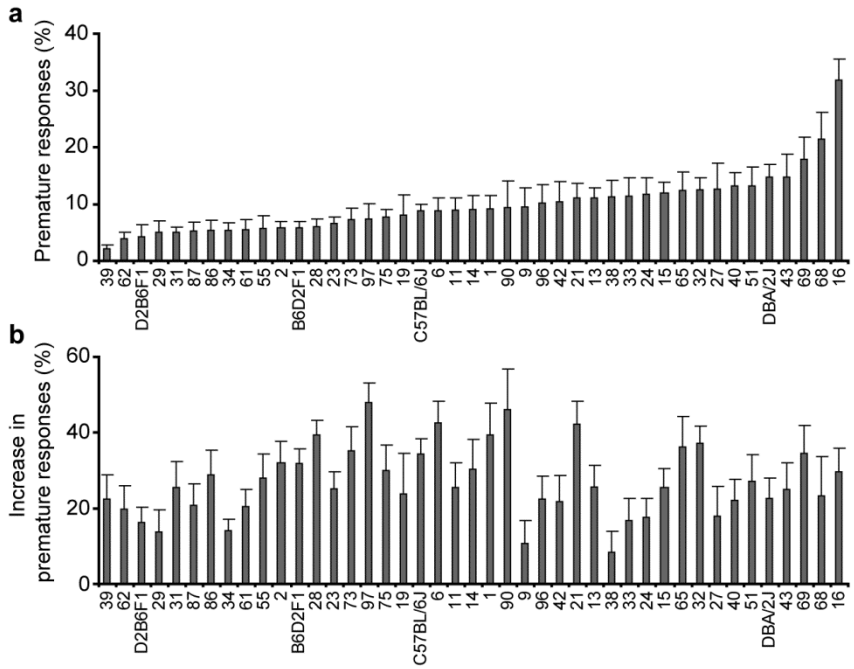

Figure 1 | (**a**) Strain differences in impulsivity, in terms of the number of premature responses in the 5-CSRTT. (**b**) During a session with an extended variable ITI (5, 7.5 and 12.5 s) the number of premature responses increased in all strains compared to baseline sessions with a fixed ITI of 5 s. Except for BXD38, this increase was significant for all strains.

QTL analysis of impulsivity and identification of candidate genes

A significant QTL was detected for impulsivity (**Fig. 2a**), with an additive effect in terms of premature responses of 2.9 %. A one-LOD drop-off confidence interval implicated 36 genes within the impulsivity QTL (**Supplementary Table 2**). Analysis of publicly available SNP data indicated the presence of non-synonymous mutations in 4 genes in the QTL (**Fig. 2b**; Ppyr1, Mmrn2, Wapal and 4930474N05Rik). In addition, analysis of adult gene expression data of prefrontal cortex tissue indicated that the correlation with a probe set for Grid1 was significant (Pearson $|r| > 0.423$, $0.01 < P < 0.05$). However, this correlation did not withstand Bonferroni correction for multiple testing (**Supplementary Table 2**).

We used the genes in the mouse QTL to guide a SNP analysis in the human data set. A total of 438 SNPs was selected from the GAIN dataset that were located in or around the human homologues of murine genes located within the one-LOD drop-off confidence interval of the impulsivity QTL. The SNP with the lowest P-value for genetic association with ADHD diagnosis is reported for each gene in **Supplementary Table 2**. After Bonferroni correction for multiple testing, the threshold for significance was set at $P < 1.14 * 10^{-4}$, indicating that none of the observed association signals could withstand correction for multiple testing indicating that even the most significant SNP in Nrg3 ($P = 0.0009$) should be considered suggestive.

Genetic correlation of impulsivity with measures of attention

Impulsivity significantly correlated (**Table 1**) with response accuracy ($P < 0.001$) but not with response variability (**Fig. 3**). No significant genetic correlations with

Table 1 | Genetic effect size and relation to impulsivity of other 5-CSRTT-related measures.

	Genetic effect size	Pearson correlation with impulsivity (r)
Acquisition		
Number of required training sessions	0.14	-0.19
Attention and impulsivity		
Response accuracy (%)	0.08	**-0.57****
Response variability (s)	0.07	0.27
Premature responses (%)	0.12	Na
Other 5-CSRTT parameters		
Number of correct responses	0.11	-0.16
Number of incorrect responses	0.05	**0.50****
Mean of correct latencies (s)	0.11	-0.09
Omission errors (%)	0.11	-0.16
Mean of magazine latencies (s)	0.05	-0.19
Body weight		
Body weight before food restriction (g)	0.30	0.07
Relative body weight during task (%)	0.03	0.07

Significant correlation (bold**), *P* < 0.001.

other frequently reported 5-CSRTT task parameters were detected with impulsivity, except for the number of incorrect responses (**Table 1**). Initial body weight before food restriction and the relative body weight (93.4 ± 0.04 % of initial body weight) during the weeks of basal 5-CSRTT training did not correlate with impulsivity, suggesting that the food regime was well-standardized across strains and did not confound observed strain differences in impulsivity.

Discussion

In this study, we observed a gradual distribution of significant differences in impulsivity among BXD recombinant inbred strains that transgressed beyond the phenotypes of the founder strains, C57BL/6J and DBA/2J. This suggested the contribution of multiple genetic loci to this phenotype, of which we detected one significant locus one on chromosome 14.

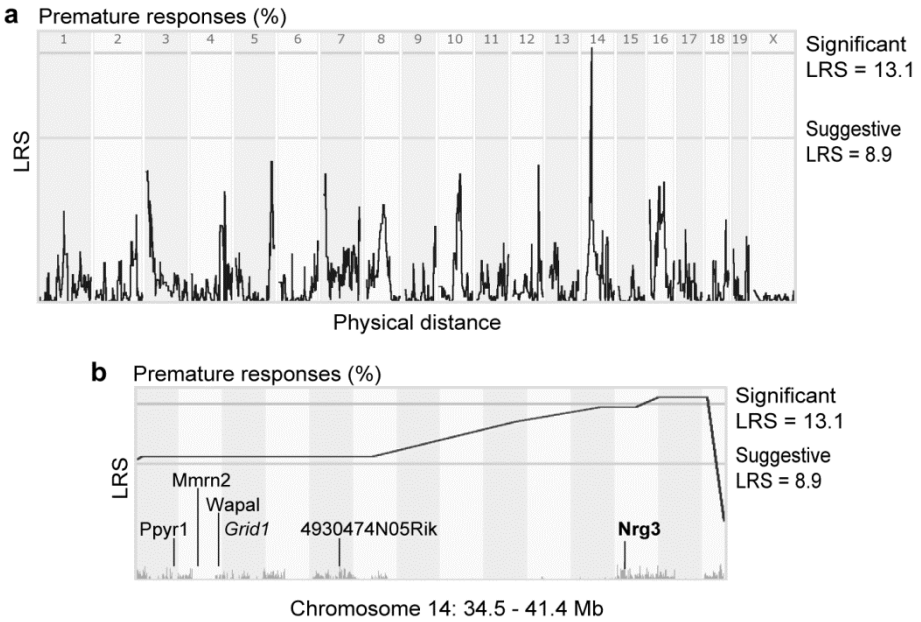

Figure 2 | Significant QTL for impulsivity. The LRS score (*y*-axis) quantifies the relation between genomic markers (*x*-axis) and the trait. The threshold for significance (P = 0.05) and suggestive significance (P = 0.63) is indicated. (**a**) Genome-wide significance was reached at a locus on chromosome 14 for impulsivity. (**b**) The magnified view displays the one-LOD drop off confidence interval of the QTL. Genes harboring non-synonymous SNPs (regular font), genes of which the expression (www.Genenetwork.org) correlated with the respective trait (*italics*) and the gene harboring the SNP with the strongest association with ADHD diagnosis (bold) are indicated. The height of the grey markers placed on the x-axis displays the number of SNPs between C57BL/6J and DBA/2J at each locus as presented in WebQTL (www.Genenetwork.org).

Candidate gene approach

This novel QTL for impulsivity did not harbor genes that have been previously associated with impulsivity or with ADHD in general. In order to narrow down the number of candidate genes in the QTL region we took three steps. First, we searched for non-synonymous mutations in these genes in existing databases. At least three genes (Ppyr1, Mmrn2 and 4930474N05Rik) harbor mutations that result in the exchange with amino acids of different polarity or charge, potentially altering the activity of the protein. In depth analysis of the effect of these mutations could indicate whether these mutations are causal to the observed behavioral phenotype.

Second, we investigated the correlation between the respective trait and the expression level of these genes in mouse adult prefrontal cortex, a brain region involved in impulsivity (Muir *et al.*, 1996). A few marginally significant correlations were observed that did not withstand correction for multiple testing. This obviously does not exclude the possibility that the causal mutation in the QTL region has a causal effect through changing the expression level of a gene. The effect on gene expression could be located in a different brain region, a subregion of the mPFC, may be cell type specific, or exert its effect during development. Third, since increased impulsivity is a core symptom in ADHD, we looked for association of SNPs in homologous human genes with ADHD diagnosis. The SNP with the lowest probability was located in Nrg3, and, resulting from bootstrap analysis, this association should be interpreted as suggestive. Interestingly, this gene has been associated with schizophrenia (Morar *et al.*, 2010), and recent evidence indicates that patients with schizophrenia show inhibitory control deficits in a stop signal reaction time task (Lipszyc & Schachar, 2010; Nolan *et al.*, 2010).

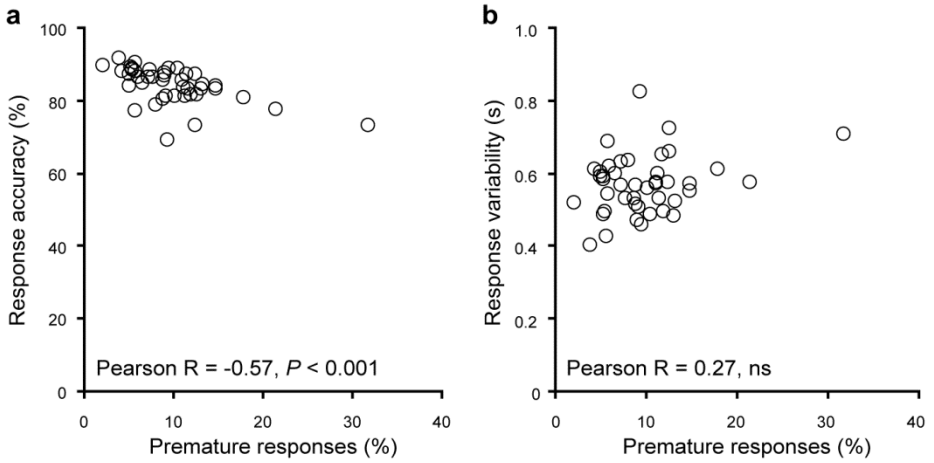

Figure 3 | Impulsivity and attention (**a**) Impulsivity (premature responses) correlated significantly with response accuracy, the traditional measure of attention in the 5-CSRTT. (**b**) The coefficient of correlation between impulsivity and response variability was low and not significant ($P = 0.07$).

Genetic correlations among measures impulsivity and attention

The negative correlation observed between impulsivity and response accuracy in the 5-CSRTT has been reported before in rats (Blondeau & Dellu-Hagedorn, 2007). Our analysis indicated that impulsivity correlated significantly with the number of incorrect responses but not with the number of correct responses, indicating that the relation between response accuracy and impulsivity depends on the propensity to make incorrect responses. This may be interpreted along a mechanism of inhibitory control. In trials in which the location of the 1 s stimulus was not detected properly, low impulsive strains may refrain from responding, whereas impulsive strains tend to make an incorrect response. As such, response accuracy does not only depend on attentional processes, but also on inhibitory control mechanisms. This may explain why impulsivity did not correlate with the other measure of attention, response variability, since the latter only takes into account correct responses and not incorrect responses. The genetic independence of impulsivity (chromosome 14), and accuracy and response variability (chromosome 16; see **chapter 5**) indicated that these measures are instrumental in disentangling inhibitory control and attentional processes, and symptomatic levels of impulsivity and response variability may result from different molecular pathways.

Taken together, despite various bioinformatics strategies, additional experiments are required to narrow down the size of the loci (fine mapping) or that manipulate the function of the candidate genes to unequivocally identify the causative genes. Future studies, identifying these genes within the QTL will help to understand the molecular pathway that contributes to impulsivity.

Acknowledgements

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Supplementary data

Supplementary Table 1 | Number of mice tested per strain.

	Tested	Excluded	Baseline	Variable ITI
BXD1	11	0	11	10
BXD2	27	-8	19	19
BXD5	10	all		
BXD6	25	-1	24	24
BXD8	14	all		
BXD9	9	0	9	9
BXD11	14	0	14	14
BXD13	14	-3	11	10
BXD14	11	0	11	11
BXD15	23	-1	22	21
BXD16	23	-2	21	18
BXD19	7	0	7	7
BXD21	12	0	12	12
BXD23	16	0	16	16
BXD24	13	-1	12	12
BXD27	9	-3	6	6
BXD28	12	-3	9	9
BXD29	14	0	14	14
BXD31	12	0	12	12
BXD32	27	-3	24	24
BXD33	14	0	14	14
BXD34	13	0	13	13
BXD38	9	0	9	9
BXD39	14	0	14	14
BXD40	26	-5	21	21
BXD42	12	0	12	12
BXD43	15	0	15	15
BXD51	12	0	12	12
BXD55	19	-1	18	18
BXD61	10	0	10	10
BXD62	12	0	12	12
BXD65	12	-3	9	9
BXD68	12	0	12	12
BXD69	12	0	12	12
BXD73	14	0	14	14
BXD75	12	0	12	12
BXD86	13	-1	12	12
BXD87	12	0	12	12
BXD90	12	-5	7	7
BXD96	12	0	12	12
BXD97	6	0	6	6
C57BL/6J	35	-1	34	34
B6D2F1	12	0	12	12
D2B6F1	11	-1	10	10
DBA/2J	20	0	20	20
Total	**654**	**-66**	**588**	**582**

Indicated is the number of mice for each strain that entered testing in 5-CSRTT protocol, the number of mice that was excluded according to criteria, the number of mice that were used to calculate the strain mean baseline performance (SD of 1.0 s and an ITI of 5 s), and the number of mice that were used to calculate the strain performance during the session with a variable ITI (5, 7.5 and 12.5 s).

Supplementary Table 2 | Genes located within the confidence interval of the impulsivity QTL on chromosome 14.

GeneSymbol	Transcription start site	SNP id (amino acid substitution)	Probe set with highest correlation	Pearson R (n = 22)	Human Homologue	Human SNP	p-value of ADHD association
AK076784	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930503F20 product:unclassifiable, full insert sequence.						
AK019667	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930503F20 product:unclassifiable, full insert sequence.						
Gdf10	bone morphogenetic protein 3B precursor		1424007_at	-0.215	GDF10	rs7093975	0.0278
Gdf2	growth/differentiation factor 2 precursor		1425882_at	0.215	GDF2	rs11204215	0.1010
Rbp3	retinol-binding protein 3 precursor		1457855_at	0.368	RBP3	rs2070706	0.3144
Zfp488	zinc finger protein 488				ZNF488	rs7071684	0.2128
EG432838	hypothetical protein LOC432838						
AK041587	Mus musculus 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A630023A22 product:unclassifiable, full insert sequence.						
Antxrl	anthrax toxin receptor-like precursor						
Anxa8	annexin A8		1417732_at	0.345	ANXA8L1		
Ppyr1	neuropeptide Y receptor type 4	rs13482131 (R/Q)# rs51067289 (P/S)#	1422271_at	0.186	PPYR1		
Syt15	synaptotagmin-15				SYT15		
3110001K24Rik	Fam35a family with sequence similarity 35, member A		1437654_at	0.176	FAM35A	rs11202332	0.3661
Glud1	glutamate dehydrogenase 1, mitochondrial		1416209_at	-0.114	GLUD1	rs2296063	0.4236
2200001I15Rik	Fam25c family with sequence similarity 25, member C		1437019_at	0.014	FAM25B		
Sncg	gamma-synuclein		1417788_at	-0.156	SNCG	rs9420407	0.2691
Mmrn2	multimerin-2 precursor	rs47039688 (G/S)# rs50165120 (L/I)# rs51174312 (D/E)	1437123_at	-0.280	MMRN2	rs34587013	0.6573
Bmpr1a	bone morphogenetic protein receptor type-1A		1451729_at	0.312	BMPR1A	rs4934265	0.1299
9230112D13Rik	9230112D13Rik RIKEN cDNA 9230112D13 gene		1431657_at	0.212			
Ldb3	LIM domain-binding protein 3 isoform e		1445121_at	-0.193	LDB3	rs6586023	0.0012
Opn4	melanopsin isoform 1		1421584_at	0.335	OPN4	rs2736689	0.3252
Wapal	wings apart-like protein homolog	rs30252573 (A/V)	1434835_at	0.100	WAPAL	rs17422850	0.0569
Grid1	glutamate receptor delta-1 subunit precursor		1445779_at	0.429*	GRID1	rs2352461	0.0056
4930596D02Rik	hypothetical protein LOC239036						

Supplementary Table 2. (*Continued*)

4930474N05Rik	hypothetical protein LOC218921	rs46605863 (H/P)#					
Gcap14	granule cell antiserum positive 14 isoform 2		1431972_a_at	0.388	KIAA1128		
Rgr	RPE-retinal G protein-coupled receptor		1422832_at	0.303	RGR	rs4244948	0.0136
Lrrc21	leucine-rich repeat, immunoglobulin-like domain		1441110_at	-0.152	LRIT1	rs6585848	0.1625
Lrrc22	leucine-rich repeat, immunoglobulin-like domain		1443940_at	-0.025	LRIT2	rs11200925	0.2038
Pcdh21	cadherin-related family member 1 precursor		1418304_at	-0.359	PCDH21	rs7099098	0.0039
AK012157	Mus musculus 10 days embryo whole body cDNA, RIKEN full-length enriched library, clone:2610528A11 product:unclassifiable, full insert sequence.						
BC049685	Mus musculus 10 days embryo whole body cDNA, RIKEN full-length enriched library, clone:2610528A11 product:unclassifiable, full insert sequence.						
Ghitm	growth hormone-inducible transmembrane protein		1447504_at	-0.347	GHITM	rs2306321	0.0117
Nrg3	pro-neuregulin-3, membrane-bound isoform isoform		1442950_at	0.140	NRG3	rs12358612	0.0009
AK133078	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930568D09 product:unclassifiable, full insert sequence.						
AK007151	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:1700109I08 product:unclassifiable, full insert sequence.						

Indicated is the mouse gene symbol, the transcription start site according to genome build 37 (mm9), the presence and identity of non-synonymous SNPs, the probe set identifier on the affymatrix microarray with the highest Pearson correlation and its correlation coefficient for the 36 genes within the impulsivity confidence interval. Human SNPs were selected in homologous human genes, and for each human gene the SNP with the strongest association with ADHD diagnosis its identifier and respective p-value are displayed. Indicated are genes with SNPs that lead amino acid substitutions with different polarity or charge (#), and with significant (*) correlation between expression in prefrontal cortex and the percentage premature responses ($0.01 < P < 0.05$). However, the indicated expression correlations did not withstand correction for multiple testing (36 correlations tested, Bonferroni corrected $P < 1.4 \times 10^{-3}$).