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Chapter 5

Independent genetic loci for sensorimotor gating and attentional performance in BXD recombinant inbred strains

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Abstract

A startle reflex in response to an intense acoustic stimulus is inhibited when a barely detectable pulse precedes the startle stimulus by 30 – 500 ms. It has been theorized that this phenomenon, named prepulse inhibition (PPI) of a startle response, is an automatic early-stage gating process contributing to the ability to focus attention. Deficits in PPI may therefore contribute to deficits in attentional processing. Both deficits are observed in schizophrenia spectrum disorders. Here, we investigated whether there is overlap in genetic control of PPI and attentional processing phenotypes in the panel of BXD recombinant inbred strains of mice. Using an individually-titrated prepulse intensity to handle differences in perceived prepulse intensities among strains, we identified a significant QTL for PPI at the mid-distal end of chromosome 17. A measure of attentional processing in the 5-CSRTT, response variability, mapped to a different locus on proximal-mid chromosome 16. In addition, the estimated genetic and environmental correlations between PPI and several attentional phenotypes were low and not significant. Taken together, the observation of separate genetic loci for PPI and attention and the absence of genetic and environmental correlations indicate that differences in sensorimotor gating do not contribute to differences in attentional performance. Therefore, it is worth pursuing the causative genes residing in both attention and PPI QTL as these may contribute to separate molecular pathways implicated in neuropsychiatric diseases, such as schizophrenia.

Introduction

The expression of a startle reflex in response to an intense acoustic stimulus is markedly reduced when preceded by a non-startling stimulus (Graham, 1975), a phenomenon called prepulse inhibition (PPI). The dominant theory of PPI is that gating mechanisms dampen processing of new disruptive input, thereby protecting processing of the prepulse stimulus (Graham, 1975). This bottom-up control over information processing is thought to contribute to the ability to focus attention on the most salient aspects of the stimulus-laden environment (Braff & Geyer, 1990; Braff *et al.*, 2001). Besides this conceptual relation between PPI and attentional processing, there is evidence from patients with schizophrenia spectrum disorders, in which deficits in PPI (for review see Braff *et al.*, 2001) and attentional performance (Nieuwenstein *et al.*, 2001) co-occur. Given the fact that both PPI and attentional performance are influenced by genetic factors (Anokhin *et al.*, 2003; Greenwood *et al.*, 2007; Groot *et al.*, 2004), this questions to what extent PPI and attentional processing might be influenced by common genetic factors.

Human PPI and attention tasks have excellent translational rodent counterparts. Rodent PPI assays show high predictive and construct validity with respect to schizophrenia (Geyer *et al.*, 2001; Powell *et al.*, 2009). The human continuous performance task (CPT) of attentional processing (CPT; Beck *et al.*, 1956) has been adapted for rodents into the 5-choice-CP task (Young *et al.*, 2009a), and the

widely used 5-choice serial reaction time task (5-CSRTT; Robbins, 2002). In the 5-CSRTT, response accuracy is the primary measure of attention. Intra-individual variability in correct response latencies (response variability hereafter) has been recognized as measure of lapses in attention (Castellanos & Tannock, 2002; Leth-Steensen *et al.*, 2000; Loos *et al.*, 2010b; Sabol *et al.*, 2003; Sergeant & van der Meere, 1990) and can also be measured in the 5-CSRTT.
To investigate whether PPI and attention phenotypes map to the same genetic loci, we employed a panel of recombinant inbred strains of mice, which are derived from an intercross of C57BL/6J and DBA/2J (BXD strains; Peirce *et al.*, 2004). These strains have previously been used to detect QTL underlying PPI, (Hitzemann *et al.*, 2001; McCaughran *et al.*, 1999; Willott *et al.*, 2003), but not attentional performance. In addition, inbred strains of mice can be used to approximate genetic and environmental correlations between PPI and attentional phenotypes (Loos *et al.*, 2009). To identify candidate genes in mouse QTL potentially influencing PPI and attention, we used brain gene expression data of BXD strains.

Materials and Methods

Animals

Parental and BXD lines were received from Jackson Laboratories, or from Oak Ridge Laboratory (BXD43, BXD51, BXD61, BXD62, BXD65, BXD68, BXD69, BXD73, BXD75, BXD87, BXD90), and were bred by the Neuro-BSIK consortium. Male seven-week-old 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 with food (Teklad 2018, Harlan Laboratories, Horst, The Netherlands) and water *ad libitum*. After one week of habituation, body weights were recorded and mice were subjected to a single 45 min session in the startle response chambers. Over the subsequent 6 days, food was limited to gradually decrease body weights to 90 – 95% of their initial body weight, before daily training in operant cages commenced (5 days each week, 30 min per session). Due to logistical reasons, not all mice tested in the 5-CSRTT were subjected to PPI testing. All experimental procedures were approved by the local animal research committee and complied with the European Council Directive (86/609/EEC).

Acoustic startle and prepulse inhibition

It has been well-documented that prepulse intensity strongly modifies PPI (Braff *et al.*, 2001; Plappert *et al.*, 2004; Varty *et al.*, 2001). This indicates that strain differences in perceived salience of prepulse stimuli could interfere with the calculation of PPI, and hence analyses of correlation with attentional performance. By generating an extensive acoustic startle response (ASR) curve

and determining the startle threshold (ST) for each individual mouse we aimed to control for differences in perceived intensity of prepulses when calculating PPI. In addition, a passive PPI procedure with neutral prepulse stimuli and a short prepulse to pulse stimulus onset asynchrony (SOA) was used to reduce (Bohmelt *et al.*, 1999; Dawson *et al.*, 1993; Filion *et al.*, 1993), but not prevent, (Elden & Flaten, 2003; Thorne *et al.*, 2005) the possibility of attentional processes to modify PPI.

Acoustic startle and PPI were measured during one 45 min session in four Plexiglas cylinders in ventilated sound-attenuating chambers (Med Associates, St. Albans, VT), placed on separate passive heavy vibration-free tables (Newport Corporation, Irvine, CA). During testing, a separate speaker provided white noise background of 75 dB. The intensity of the startle stimuli was calibrated with a microphone placed inside the Plexiglas cylinders in a closed chamber, while white background noise was switched off. Hence, the reported prepulse and pulse intensities are lower than the actual cumulative sound pressure level during testing (e.g., a 75 db pulse in addition to a 75 dB background noise add up to 78.01 dB). The session started with a habituation period of 5 min, followed by a total of 245 trials with pseudo randomized interval periods (5 – 15 s) consisting of 10 acoustic startle trials with white noise bursts at various intensities (65, 70, 75, 80, 85, 90, 95, 100, 105, 110, and 115 dB) and 15 prepulse inhibition trials with white noise bursts at various prepulse intensities (0, 65, 70, 75, 80, 85, 90, 95, 100 dB; startle intensity always 120 dB) in pseudo randomized order such that all 4 boxes produced startle stimuli at exactly the same time. Onset of white noise prepulse stimuli (20 ms; 1 ms programmed rise/fall time) and startle stimuli (40 ms; no programmed rise/fall time) were separated by a 60 ms interval (i.e., SOA). In each trial, the highest startle intensity peak (in relative machine units) was collected during the 100 ms interval after the startle stimulus, from which the individual mean highest startle intensity peak during the 100 ms null-period prior to prepulse stimuli was subtracted. The startle sensitivity was determined for each mouse by determining the ST at which a statistically significant startle response was measured (one-sample t-test, with average null-period as reference value). The equipment was calibrated to allow for a wide range of startle intensities, however, the force generated by some mice at the highest pulse intensities could exceed the dynamic range of the equipment (maximum of 2047 units) artificially reducing the percentage of PPI in subsequent analyses. Therefore, when the number of such censored 120 dB pulse trials was more than 33% (e.g., more than 5 out of 15) no PPI was calculated. The percentage of PPI was calculated as follows: PPI = 100 * [(mean startle intensity pulse) - (mean startle intensity pulse with prepulse) / (mean startle intensity pulse)].

5-CSRTT

Mice were trained to perform the 5-CSRTT on an individually-paced schedule in operant chambers (MEDNPW-5M, MedAssociates, St Albans, VT, USA), as

described previously (Loos *et al.*, 2010b; Loos *et al.*, 2009). During the first week, mice underwent one 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 one of the five stimulus holes was presented for a limited duration (stimulus duration). A response in the correct stimulus hole within the limited hold period of 4 s after termination of the stimulus, switched on the magazine light and delivered a food pellet. The latency between a correct response and the detection of a head entry into the magazine were recorded (magazine latency). 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, and 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)]. The response accuracy was defined as [100 × (number of correct responses) / (number of correct and incorrect responses)]. In the first 5-CSRTT session the stimulus duration was set at 16 s, and was decreased in subsequent sessions to 8, 4, 2, 1.5 and 1 s when the subject reached criterion performance (omissions < 30%, accuracy > 60%, started trials > 50) or after 10 sessions. Intra-individual variability of correct response latencies was defined by the standard deviation of the correct response latencies. In addition, the mean and the mode of correct response latencies were calculated as described previously (Loos *et al.*, 2010b). Dependent measures were calculated from the 6^{th} until the 10^{th} 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 10^{th} session, on Tuesday the stimulus duration was programmed to vary (1, 0.5 and 0.25 s; durations 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, the statistics of one-way analysis of variance (ANOVA) were reported. The effects of startle stimulus intensity on ASR were analyzed using repeated-measures ANOVA; if Mauchly's test for sphericity of data was significant, more conservative Greenhouse-Geisser corrected degrees of

freedom and resulting probability values were reported. Pearson correlations coefficients and respective probabilities were calculated across strain means, to approximate genetic correlations. Environmental correlations were estimated from scores of individual mice after subtraction of the respective strain mean, as described previously (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 using 1000 permutations, were used to assess the whether the peak of a QTL was statistically significant (genome-wide *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 databases of whole brain (UCHSC BXD Whole Brain M430 2.0 November 2006 RMA, Accession number: GN123) or prefrontal cortex tissue (Virginia Commonwealth University, BXD Prefrontal Cortex Saline Control M430 2.0 (Dec06) RMA Dataset, Accession number: GN135) were used to calculate Pearson correlations of different probesets of genes with measures of PPI and attention respectively, using GeneNetwork's online tools. For the analysis of non-synonymous mutations in genes under the QTL peak, GeneNetwork's comprehensive SNP browser was used (date: June 2010). Behavioral phenotypes of mouse mutants of candidate genes detected in our study were derived from the Mouse Genome Informatics (MGI) database and information on human disorders associated with these candidate genes was derived from the Online Mendelian Inheritance in Man (OMIM) database.

Results

Strain differences in acoustic startle and prepulse inhibition

A total of 442 mice, encompassing 39 strains, was tested for ASR and PPI, i.e., 35 BXD strains, the parental lines and the reciprocal F1 ($n \geq 6$ per strain, average 11.3 per strain). The mean startle intensity levels during the null period differed significantly among strains ($F(38,403) = 4.99$, $P < 0.001$), indicative of differences in activity between strains in the apparatus, and was used to correct startle responses intensities as described in the materials and methods.

Startle threshold. The ASR significantly increased when louder startle stimuli were applied (**Fig. 1**; strain: $F(1.91,773.42) = 1005.80$, $P < 0.001$). We observed significant strain differences in the shape of these ASR-curves (strain × startle stimulus intensity: $F(72.93, 773.42) = 9.1$, $P < 0.001$), that were at least partly explained by the threshold at which mice started showing a significant startle (ST; **Fig. 2a**). A significant difference among strains was detected for this individually determined ST ($F(38,403) = 7.44$, $P < 0.001$).

Prepulse inhibition. The variation among mice in ST could reflect differences in the perceived salience of the auditory stimuli. To control for differences among mice in perceived salience of prepulse stimuli, data of prepulse trials, in which the prepulse intensity was 10 dB lower than the ST, were taken to calculate the individually titrated PPI. In addition, we used filtering criteria (see Materials and Methods) to exclude mice with extreme startle sensitivity (ST ≤ 75 dB) or startle intensity (> 33% censored pulse trials), together excluding 3 strains for further analyses due to low n (n < 6; BXD 1, 6, 97). Significant strain differences in PPI were detected among the remaining 335 mice of 36 strains at the individually titrated prepulse intensity (**Fig 2b**; $F(35,299) = 2.50$, $P < 0.001$). Furthermore, conventional prepulse inhibition percentages were calculated at prepulse intensities ranging from 65 – 75 dB and significant strain differences were detected (65 dB: $F(35,299) = 4.70$, $P < 0.001$; 70 dB: $F(35,299) = 5.23$, $P < 0.001$; 75 dB: $F(35,299) = 5.44$, $P < 0.001$). Strains with known hearing deficits (BXD16 and BXD24; Willott & Erway, 1998) showed a decreased PPI at these conventional PPI, however, they showed PPI to the same extent as other BXD strains at the individually titrated prepulse intensity.

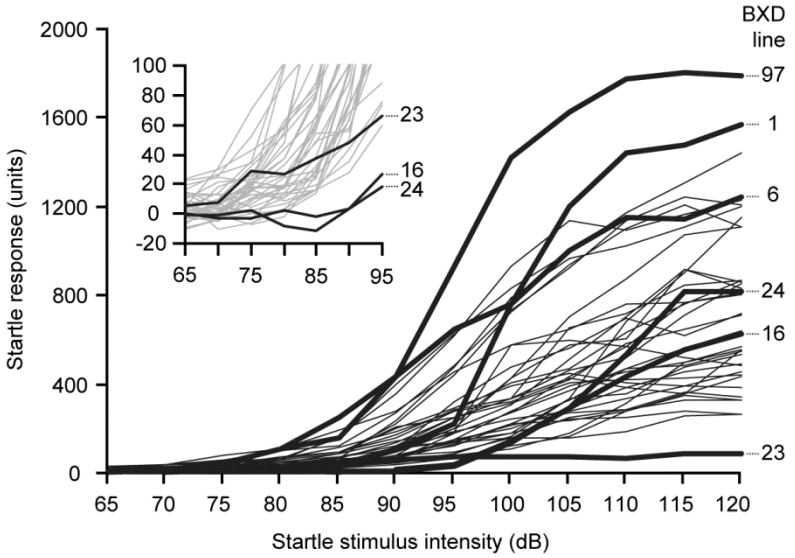

Figure 1 | Qualitative differences in the shape of ASR curves. For each strain the average ASR curve is presented. Although BXD16 and 24 show a clear startle response at 120 dB, the inset indicates impaired startle at low stimulus intensities, which is reflected in their high ST. BXD 23 shows a clear startle at low stimulus intensities, but remarkably low startle at 120 dB compared with other strains. Some strains (e.g., BXD 1, 6 and 97) show an asymptotic ASR curve due to the censoring of startle response at an upper limit of 2047 units (see M&M for inclusion criteria).

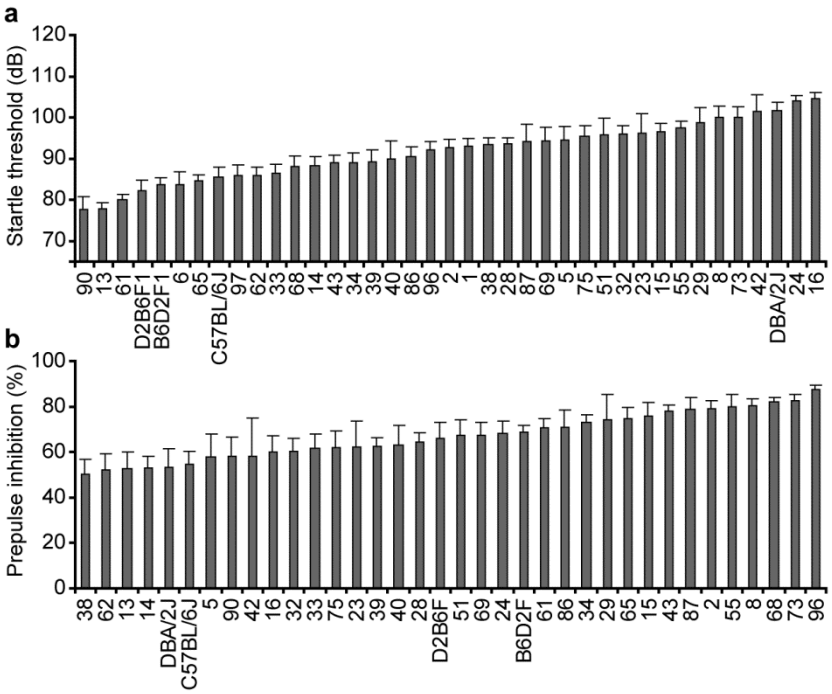

Figure 2 | Strain differences in startle sensitivity. (**a**) The startle stimulus intensity (dB; ± SEM) at which mice showed significant startle compared with the average null-period, 100 ms prior to a stimulus, was taken as startle threshold (ST). (**b**) The percentage of PPI (± SEM) was calculated for trials at the individually titrated prepulse intensity.

Strain differences in attentional performance

In total, we analyzed 5-CSRTT data of 558 mice, distributed across 41 BXD strains (on average n = 13 per strain, minimum n = 6 per strain) and the parental lines (C57BL/6J, n = 34 and DBA/2J, n = 20). Significant strain differences were detected for measures of attention; response accuracy (**Fig. 3a**; $F(42, 545) = 3.69$, $P < 0.001$), response variability (**Fig. 3b**; $F(42, 545) = 2.71$, $P < 0.001$) and errors of omission ($F(42, 545) = 4.48$, $P < 0.001$; not shown). The first two of these measures correlated significantly across strains ($r_{\text{response accuracy, response variability}} = -0.75$, n = 43, $P < 0.001$). When calculated from the scores of individual mice (i.e., after subtraction of the respective strain mean), the estimated environmental correlation between both measures was strong ($r_{\text{response accuracy, response variability}} = -0.64$, n = 588, $P < 0.001$). In contrast, errors of omission, did not correlate with response accuracy ($r_{\text{response accuracy, omissions}} = -0.06$, n = 43, n.s.) and response variability ($r_{\text{response variability, omissions}} = -0.01$, n = 43, n.s.) across strains, or across individual performance ($r_{\text{response accuracy, omissions}} = 0.04$, n = 588, ns; $r_{\text{response variability, omissions}} = 0.00$, n = 588, n.s.), indicating that this measure taps into a different aspect of 5-CSRTT performance. Although mice with potentially low motivation or motor impairment, as indicated by number of trials and magazine latencies

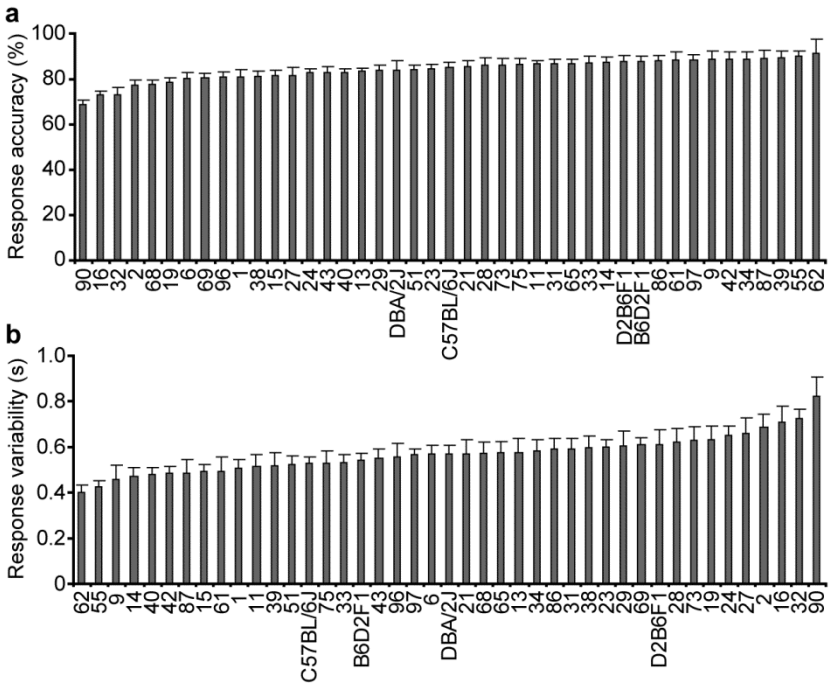

Figure 3 | Strain differences in attentional performance of BXD strains. (**a**) Response accuracy is the traditional measure of attention in the 5-CSRTT. All BXD strains showed response accuracy (± SEM) well above chance level (20%). (**b**) Displayed is the mean response variability (± SEM), a putative measure of lapses in attention.

were removed before analyses, we did observe a correlation between errors of omission and magazine latencies ($r_{\text{omissions, magazine lantencies}} = 0.47$, n = 43, $P < 0.001$). All included mice showed response accuracies above 40%. Furthermore, we observed a significant decrease in response accuracy after increasing attentional load by randomly shortening the stimulus duration (SD: $F(1,534) = 355.46$, $P < 0.001$; SD × strain: $F(42, 534) = 1.53$, $P < 0.05$), which did not reach significance in all strains (**Supplementary Fig. 1**). Together these data indicate that all strains used an attention strategy to solve the task, as opposed to a random response strategy.

QTL analysis and candidate gene analyses

Prepulse inhibition. A significant QTL was detected for the percentage of PPI at the individually titrated prepulse intensity (**Fig. 4a**) at chromosome 17 (one-LOD drop-off confidence interval: 71.10 – 75.47 Mb). The peak additive effect in terms of PPI was 7.63%, with the BL6 allele contributing to higher PPI scores. No significant QTL were detected for ASR or other measures of PPI. Suggestive QTL are presented in **Table 1**. The PPI QTL region contained 33 genes (**Supplementary Table 1**). Analyses of publicly available SNP data of

C57BL/6J and DBA/2J mice (www.genenetwork.org) indicated the presence of non-synonymous mutations in 7 of these genes (**Fig. 4a**; Myom1, Emilin2, Ndc80, Spdya, Clip4, Alk, Tct27). Analyses of adult gene expression data of whole brain tissue indicated that from these 33 genes there were significant correlations between the percentage of PPI at the individually titrated prepulse intensity and probe sets targeting three genes (**Fig. 4a**; YpeI5, Ehd3, Spast) (Pearson $|r| > 0.444$, $P < 0.05$). However, none of these correlations could withstand Bonferroni correction for multiple testing (**Supplementary Table 1**).

Measures of attention. A significant QTL was detected for response variability (**Fig. 4b**; peak additive effect 0.053 s) at chromosome 16 (one-LOD drop-off confidence interval: 19.88 – 26.55 Mb), with the BL6 allele contributing to reduced response variability (i.e., increased attentional performance). This QTL coincided with a suggestive QTL for response accuracy at approximately the same location (**Table 1**), in which the BL6 allele contributed to increased response accuracy. Other dependent 5-CSRTT measures mapped significantly (mode of correct response latencies; Chr16 (93.93 – 97.54)) or suggestively (mean of response latencies, errors of omission and magazine latencies; see **Table 1**) to loci that did not overlap the attention QTL on chromosome 16. Analyses of publically available SNP data of C57BL/6J and DBA/2J mice (www.genenetwork.org) did not indicate non-synonymous mutations within the 79 genes located in the attention QTL. Analyses of adult gene expression data of prefrontal cortex tissue indicated that correlations between response variability and probe sets targeting exons or untranslated regions of four of these genes (**Fig. 4b**; Abcc5, 2310042E22Rik, Fetub and Cldn16) were significant (Pearson $|r| > 0.423$, $0.01 < P < 0.05$). However, none of these correlations could withstand Bonferroni correction for multiple testing (**Supplementary Table 2**).

No genetic or environmental correlation between sensorimotor gating and attention

Response accuracy and response variability did not correlate significantly with the percentage of PPI at the individually titrated prepulse intensity across strain means (**Fig. 5a**). PPI at prepulse intensities ranging from 65 – 70 dB did not correlate with attention in the 5-CSRTT either, except for the correlation between accuracy and PPI at the lowest prepulse intensity of 65 dB ($r_{PPI65,\ accurate\ responses} = 0.36$). This correlation was solely due to two BXD strains with known hearing impairment (BXD16 and BXD24; (Johnson *et al.*, 2000; Willott & Erway, 1998)) and high ST (**Fig. 2b**); when these strains were excluded from correlation analyses, no significant correlation was detected anymore (**Fig. 5b**). In addition to these correlations across strain means, which approximate the genetic correlation, we calculated environmental correlations from scores of individual mice after subtraction of strain means between PPI at various prepulse intensities (individually titrated, 65, 70 and 75 dB) and attention (response accuracy and response variability). All of these environmental correlations were

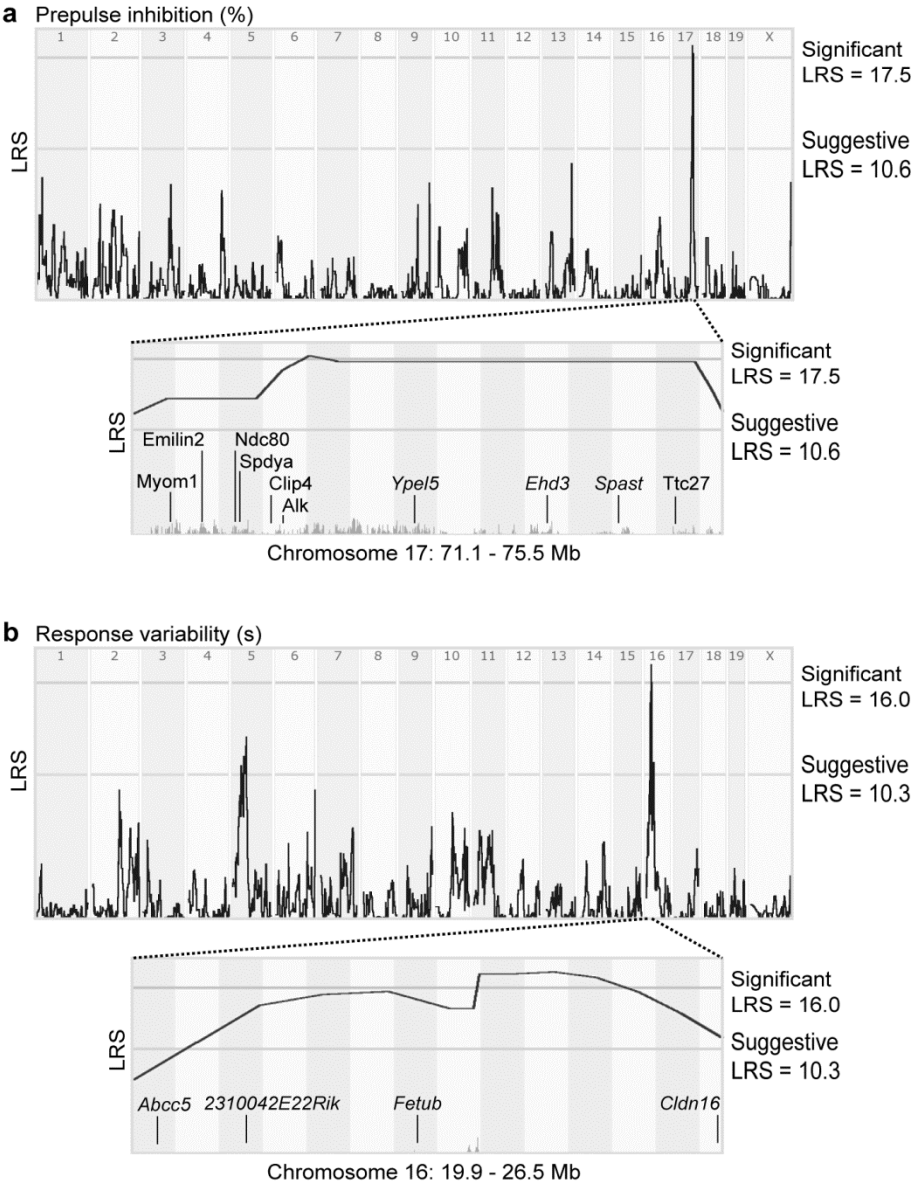

Figure 4 | Significant QTL for PPI and attention. The LRS scores (*y*-axis) quantify the relation between genomic markers (*x*-axis) and the trait. The threshold for significance (genome-wide P = 0.05) and suggestive significance (genome-wide P = 0.63) is indicated. (**a**) Genome-wide significance was reached at a locus on chromosome 17 for PPI at individually titrated prepulse intensity and (**b**) response variability at a locus on chromosome 16. The magnified views display the one-LOD drop off confidence interval of the QTL. Candidate genes potentially influencing both traits are indicated. The 7 genes harboring non-synonymous SNPs are indicated, and the 7 genes of which the expression correlated with the respective trait are indicated in *italics*. 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).

non-significant ($|r| < 0.07$, $n = 294$). Furthermore, we did not detect genetic or environmental correlations between errors of omission and PPI measures.

Discussion

QTL for sensorimotor gating

We observed substantial differences in ASR at maximum startle intensity and percentage of PPI among mouse strains, indicative of a genetic contribution to these phenotypes in line with previous studies (Hitzemann *et al.*, 2001; Hitzemann *et al.*, 2008; Leussis *et al.*, 2009; Liu *et al.*, 2003; Petryshen *et al.*, 2005; Samocha *et al.*, 2010; Watanabe *et al.*, 2007). In addition, we noted differences in the shape of ASR curves among inbred strains that we collapsed into a parameter called startle sensitivity (i.e. ST) that showed a genetic effect size of 30%. Using this ST, we were able to identify 1) mice that exhibit a startle response at stimulus intensity levels that are regularly used as prepulse stimulus (i.e., 65 – 75 dB), and 2) mice that do not show a startle response until intensity levels increased up to 115 dB. This is problematic in face of the definition of PPI; the prepulse should not elicit a startle response. Furthermore, this suggests that a prepulse with certain intensity (e.g., 70 dB) may not be equally salient to all mice, and therefore may impact the percentage of PPI. One reason for differences in perceived salience of the prepulse is the segregation of alleles that cause age-related hearing loss in the panel of BXD lines (Johnson *et al.*, 2000; Willott & Erway, 1998). As a result, strain differences in PPI at a specific prepulse intensity may reflect differences in perceived salience in addition to differences in sensorimotor gating function.

To counteract potential differences in prepulse perception, we titrated the

Table 1 | Significant and suggestive QTL of PPI and attention.

Measure	One LOD drop–off C.I. of QTL (Mb)
ASR at 120 dB	Chr3(54.56 – 57.63), Chr10(64.17–71.42), Chr12(15.66–26.75, 36.1–46.20)
Startle Threshold	Chr10(67.08–79.09), Chr16(59.7–67.89, 76.38–82.14), Chr18(34.13–39.20)
PPI at 65 dB	Chr1(108.76–126.52), Chr12(115.17–12.16), Chr13(104.62–108.47)
PPI at 70 dB	Chr1(108.76–126.52), Chr13(105.47–107.93)
PPI at 75 dB	Chr1(108.76–126.52), Chr13(106.90–107.82)
PPI at ST – 10 dB	**Chr17(71.10–75.47)**
Response accuracy	Chr2(137.49–159.19), Chr5(14.16–23.47), Chr16(23.74–27.50)[1]
Response variability	Chr5(24.39–59.04), **Chr16(19.88–26.55)**
Errors of omission	Chr14(79.83–84.04), Chr19(57.68–61.21)
Response latency mean	Chr5(29.34–40.03), Chr8(105.16–113.84), Chr12(48.41–59.05), Chr16(92.95–97.61, 17.66–26.07[1], 7.96–13.14)
Response latency mode	**Chr16(93.93–97.54),** Chr8(105.16–113.84), Chr12(48.41–59.05)
Magazine latency	Chr8(98.23–99.92), Chr13(45.77–52.73), Chr16(30.07–36.61), Chr19(16.50–21.21)

For each parameter the one LOD drop-off confidence interval is given for suggestive (genome-wide $P < 0.63$) and significant (genome-wide $P < 0.05$) QTL. QTL that reached genome wide significance are indicated in **bold**. [1]This suggestive QTL overlaps with the location of the significant response variability QTL.

prepulse intensity for individual mice to 10 dB below its ST under the assumption that such a prepulse would be perceived, but would not result in a startle response. We used the percentage of PPI elicited by these individually titrated prepulses in a genetic mapping analysis, and detected a significant QTL on mouse chromosome 17. This finding at least indicates the sensitivity of our PPI analysis method to genetic effects.

Notwithstanding procedural differences, our PPI data correlated significantly with published and unpublished PPI datasets of intersecting panels of BXD strains in WebQTL (best correlation found: $r_{PPI75\ current\ study,\ PPI85\ Philip\ et.\ al.} = 0.51$, $P < 0.05$; n = 28; record ID 11428). Despite this consistency in results, there is no overlap in significant or suggestive QTL identified by individually titrated PPI in the current study (Chr 1, 12 (~118mb), 13, 17), or conventional PPI in our study, with significant QTL detected in other studies using intercrosses of C57BL/6J and DBA/2J mice, such as Chr 3, 5, 11 and 16 (Hitzemann *et al.*, 2001; Hitzemann *et al.*, 2008; Liu *et al.*, 2003). Also, no overlap between these QTL and QTL identified using intercrosses of other mouse strains were identified, e.g., Chr 4, 10, 11, 12, 16 (~73 Mb) and Y (Leussis *et al.*, 2009; Petryshen *et al.*, 2005; Samocha *et al.*, 2010; Watanabe *et al.*, 2007). Apart from differences in the experimental set-up between all these studies, the lack of overlap with our study might be due to differences in the programmed interval between prepulse and pulse onset in this study (60 ms) compared to all previous studies (100 ms), a factor that is well-known to modulate PPI (Braff *et al.*, 2001; Plappert *et al.*, 2004; Varty *et al.*, 2001).

In addition to the numerous SNPs surrounding each gene (**Fig. 4a**, *x*-axis) within the confidence interval of the PPI QTL on chromosome 17 that may affect gene expression, at least 7 genes contained non-synonymous SNPs. Adult whole brain gene expression levels of 3 genes showed marginally significant correlations with the PPI phenotype that did not withstand correction for multiple testing. Nevertheless, significant gene expression correlation may reside in specific brain regions, or at different developmental stages. None of these 10 genes or any other gene in the QTL is implicated in a mouse behavioral phenotype related to sensorimotor gating in the MGI database, or a human inherited disorder involving deficits in sensorimotor gating in the OMIM database.

QTL for attentional processing

The human continuous performance task of attentional processing (CPT; Beck *et al.*, 1956) has been adapted for rodents into the widely used 5-CSRTT (Robbins, 2002). Apart from the 5-CSRTT, as used in this study, a 5-choice CPT was developed to increase similarity to the human CPT (Young *et al.*, 2009a), Although no differences in attentional performance were detected between C57BL/6J and DBA/2J, in line with previous reports in the 5-CSRTT and 5-choice CPT (Loos *et al.*, 2010b; Young *et al.*, 2009a), we observed significant differences among BXD recombinant inbred strains that transgressed beyond the

phenotypes of the founders. This suggested the contribution of multiple genetic loci to these phenotypes, of which we detected a significant one on chromosome 16 for response variability. We observed a strong correlation between response accuracy - the most frequently used measure of attentional processing (Robbins, 2002) - and a novel putative measure of lapses in attention in the 5-CSRTT, i.e., response variability. This correlation is not due to mathematical dependence, since response accuracy is calculated from numbers of responses, whereas response variability is calculated from latencies of these responses. Moreover, the significant chromosome 16 QTL for response variability coincided with a suggestive QTL for response accuracy. Together this supports the idea that these different measures share underlying mechanisms, which would be in line with the coincidence of reduced CPT performance and increased response variability in humans (Bidwell *et al.*, 2007; Klein *et al.*, 2006). Other 5-CSRTT measures that, in addition to attentional performance, may reflect motor function and/or motivation mapped significantly or suggestively to loci that did not overlap with attention QTL on chromosome 16, emphasizing the specificity of the QTL with respect to attention.

Errors of omission did not correlate with response accuracy and response variability, and did not map to the chromosome 16 locus. Errors of omission may capture aspects of attentional processing, provided that other task parameters can exclude motivational deficits or motor impairments (Guillem *et al.*, 2011; Robbins, 2002). Nonetheless, the observed positive correlation between errors of omission and magazine latencies suggests that this measure in the current study may especially reflect motivational/motor aspects of task performance.

None of the 79 genes located in the significant QTL for response variability on chromosome 16 harbored non-synonymous SNPs. Although this region shows relatively few SNPs between the C57BL/6J and DBA/2J founder strains (**Fig. 4b**, *x*-axis), the ones that are present can affect gene expression. Analysis of correlation with adult gene expression data obtained from the prefrontal cortex, a region implicated in attentional performance in rodents in the 5-CSRTT (Muir *et al.*, 1996) indicated marginally significant correlations with 4 genes, which did not withstand correction for multiple testing. Nevertheless, significant gene expression correlation may reside in other brain regions or at different

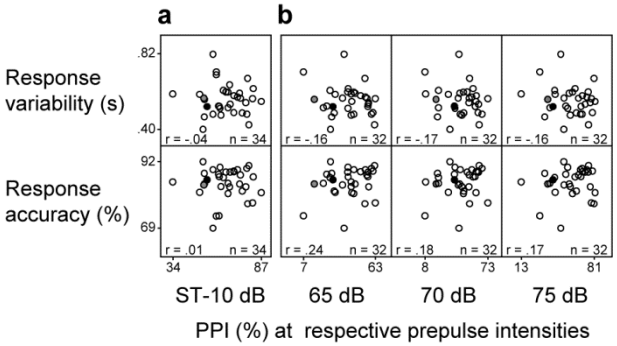

Figure 5 | No significant correlation between PPI and attention.(**a**) Response accuracy and response variability did not correlate with PPI at the individually titrated prepulse intensity, and (**b**) with conventional PPI at prepulse intensities of 65 – 75 dB. Founder lines are indicated (C57BL/6J in black, DBA/2J in grey).

developmental stages. According to the MGI database only the gene Chrd has been associated with behavioral phenotypes in mice that could remotely be relevant for attentional performance, i.e. spatial memory (Sun *et al.*, 2007). None of the genes in the QTL significantly associated with a human inherited disorder specifically related to attentional performance in the OMIM database.

Sensorimotor gating and attentional processing do not share genetic factors

An important outcome of this study was that the significant QTL for response variability in the 5-CSRTT did not map to a locus significantly linked to PPI in our data, nor to a PPI locus in any previously published study (Hitzemann *et al.*, 2001; Leussis *et al.*, 2009; Liu *et al.*, 2003; Petryshen *et al.*, 2005; Samocha *et al.*, 2010; Watanabe *et al.*, 2007) except one (Hitzemann *et al.*, 2008). However, the direction of QTL for PPI in the latter study was of the opposite effect; the B6 allele decreased PPI, while it increased attention performance in the current study (i.e., it decreased response variability). Furthermore, neither of the measures of attention correlated significantly with any measure of PPI in our study.

Current theories suggest a bidirectional relation between PPI and attentional performance. Firstly, the gating mechanisms responsible for PPI may enhance attention performance by filtering out excess or irrelevant stimuli in bottom-up flow of information (Braff *et al.*, 2001; Graham, 1975). Secondly, levels of PPI are increased when humans or rodents selectively attend to prepulse stimuli, indicative of cognitive top-down control over sensorimotor gating (Bohmelt *et al.*, 1999; Dawson *et al.*, 1993; Elden & Flaten, 2003; Filion *et al.*, 1993; Thorne *et al.*, 2005). Hence, there is a clear relation between attentional performance and PPI in tasks that employ top-down modulation of PPI (Dawson *et al.*, 1993; Dawson *et al.*, 2000; Scholes & Martin-Iverson, 2009, 2010). In the current study, we investigated the bottom-up relation between PPI and attention. We used passive prepulse stimuli, in contrast to rodent studies that focus on top-down modulation of PPI by pairing foot shocks with prepulse stimuli prior to the actual PPI task (Li *et al.*, 2009). Furthermore, the short prepulse to pulse interval (SOA of 60 ms) may reduce the possibility that attentional processes modify PPI (Braff *et al.*, 2001; Li *et al.*, 2009), as was observed in several studies (Bohmelt *et al.*, 1999; Dawson *et al.*, 1993; Filion *et al.*, 1993), but not all (Elden & Flaten, 2003; Thorne *et al.*, 2005). Because of the use of neutral prepulse stimuli and the short prepulse-pulse interval, PPI in the current study is not expected to be influenced by top-down attentional modification. The lack of correlation between PPI scores and attentional performance therefore suggest that the bottom-up gating mechanisms involved in PPI do not contribute to attention performance in the 5-CSRTT.

The absence of both a genetic and environmental correlation between the passive PPI procedure and attention are in line with studies in humans demonstrating no significant relation between measures of attention and PPI scores in passive PPI tasks (Bitsios & Giakoumaki, 2005; Greenwood *et al.*, 2007; Swerdlow *et al.*,

1995). In further support of this, mutant mice showing deficits in attention did not show altered PPI in passive procedures (i.e. Acnb2: Guillem *et al.*, 2011; Drd4: Young *et al.*, 2011). However, in a panel of 30 C57BL6J mice, Bitanihirwe et al. found a significant correlation between individual attentional performance in a 2-choice task and PPI scores in a PPI protocol that differed in the SOA used (100 ms instead of 60 ms in the current study, Bitanihirwe *et al.*, 2011). As previously proposed (Scholes & Martin-Iverson, 2009), attention to an auditory stimulus is not absent in a passive PPI task, it is just unmeasured, meaning that mice may indeed be actively listening out to the prepulse stimuli. Only when the time interval between prepulse and pulse allows, differences in PPI scores could be explained by differences in attentional processes between individual mice. Hence, whereas our study focused on the bottom-up relation between PPI and attentional performance, the study by Bitanihirwe and coworkers may also have studied top-down attentional modulation of PPI. Furthermore, within-strain variability in inbred strains results from (largely unknown) factors in the environment of individual mice that can be specific to the strain used. Hence, the discrepancy between both studies could result from strain-specific environmental factors present in the latter study that affected C57BL/6J mice but not the large cohort of BXD mice in the current study. Taken together, the coincidence of reduced PPI and deficits in attention may not be due to modification of attentional processes by PPI, but rather by impaired top-down modification of PPI by attentional processes (Braff *et al.*, 2001; Li *et al.*, 2009).

In conclusion, the differences in startle sensitivity among strains in the current study warrant future PPI studies in genetically diverse populations to take potential differences in perceived prepulse salience into account. Additional mapping studies are required to reproduce and fine-map both the PPI and response variability QTL to pinpoint the causative genes. Attentional performance does not appear to be modified by sensorimotor gating. Therefore, it is worth pursuing causative genes residing in both attention and PPI QTL to better understand the separate mechanisms that contribute to diseases such as schizophrenia.

Acknowledgments

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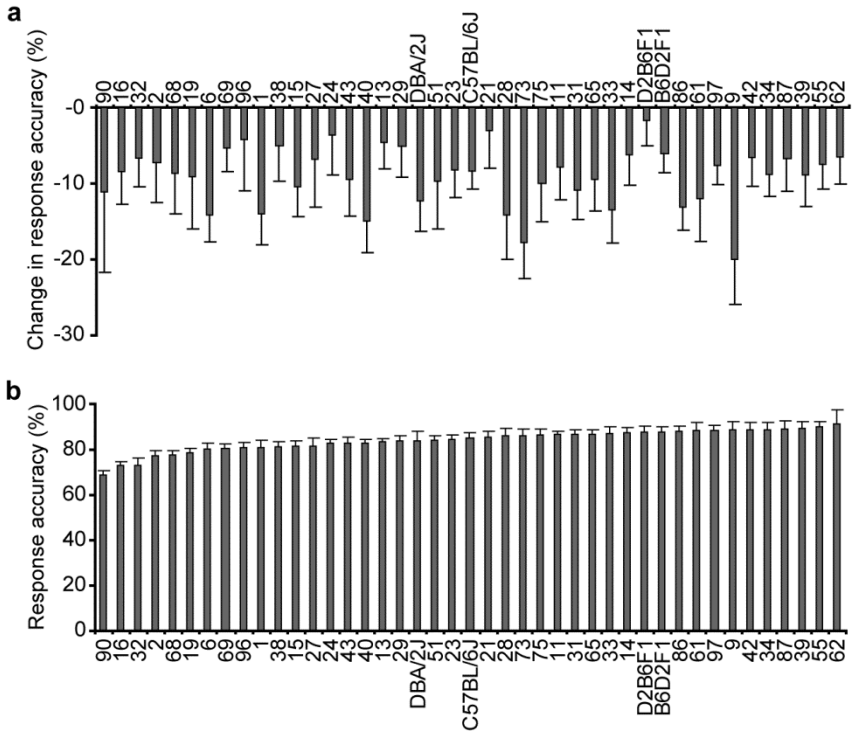
Supplementary data



Supplementary Figure 1 | Decrease in response accuracy during a session with variable stimulus duration. (a) Decrease in response accuracy (± SEM) during the session with fixed randomly variable stimulus duration (0.25, 0.5 and 1 s) compared with the response accuracy during the baseline sessions. Response accuracy decreased in all strains, but not significantly in BXD 19, 21, 24, 27, 38, 42, 69, 90, 96 and D2B6F1. From left to right, strains are ranked on increasing response accuracy (**b**), as depicted in **Figure 3a** in the main text.

Supplementary Table 1 | Genes located within the confidence interval of the PPI QTL on chromosome 17.

GeneSymbol	Description	Non-synonymous mutations	Probe set with highest correlation	Pearson R (n = 20)
Dlgap1	disks large-associated protein 1 isoform 2		1438098_at	0.403
mKIAA4162 (Dlgap1)	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4933422O14 product:DISKS LARGE-ASSOCIATED PROTEIN 1 (DAP-1) (GUANYLATE KINASE-ASSOCIATED PROTEIN) (SAP90/PSD-95-ASSOCIATED PROTEIN 1) (SAPAP1) (PSD-95/SAP90 BINDING PROTEIN 1) (FRAGMENT) homolog [Mus musculus], full insert sequence.			
Tgif1	homeobox protein TGIF1 isoform c		1444752_at	-0.131
Mylc2b	myosin regulatory light chain 12B		1428609_at	0.403
2900073G15Rik (Myl12a)	myosin light chain, regulatory B-like		1450013_at	-0.269
Myom1	myomesin-1 isoform 2	MRS11808222 MRS11808398	1420693_at	0.214
Lpin2	phosphatidate phosphatase LPIN2 isoform 2		1460290_at	-0.223
Emilin2	EMILIN-2 precursor	rs33256687 MRS11808658 MRS11808663 rs33550769 rs33068573 MRS11808782	1435264_at	0.163
mKIAA0650 (Smchd1)	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4931400A14 product:hypothetical protein, full insert sequence.			
Smchd1	structural maintenance of chromosomes flexible		1442497_at	0.348
Ndc80	kinetochore protein NDC80 homolog	rs33625632		
Spdya	speedy homolog A (Xenopus laevis)	rs33645336		
mKIAA0007 (Wdr43)	Mus musculus 10 days embryo whole body cDNA, RIKEN full-length enriched library, clone:2610318G08 product:KIAA0007 PROTEIN (FRAGMENT) homolog [Homo sapiens], full insert sequence.			
Wdr43	WD repeat-containing protein 43		1428390_at	0.025
4632412N22Rik (Fam179a)	hypothetical protein LOC320159; family with sequence similarity 179, member A		1440898_at	-0.144
Clip4	Mus musculus adult male thymus cDNA, RIKEN full-length enriched library, clone:5830409B12 product:weakly similar to CLIP-170-RELATED PROTEIN [Homo sapiens], full insert sequence.	MRS11809565 MRS11809573	1431382_a_at	-0.058

Supplementary Table 1. (*Continued*)

Alk	ALK tyrosine kinase receptor precursor	MRS11811254 MRS11811903 MRS11811905 MRS11811906	1449987_at	0.023
Ypel5	protein yippee-like 5		1451196_at	-0.602[#]
Lbh	protein LBH		1451629_at	-0.216
Lycat	lysocardiolipin acyltransferase 1		1434690_at	-0.004
Capn13	calpain-13		1437960_at	-0.065
Galnt14	polypeptide N-acetylgalactosaminyltransferase		1453630_at	-0.155
Ehd3	EH domain-containing protein 3		1417235_at	0.468[#]
Xdh	SubName: Full=Putative uncharacterized protein;		1451006_at	0.289
Srd5a2	3-oxo-5-alpha-steroid 4-dehydrogenase 2		1422960_at	-0.021
Memo1	protein MEMO1		1449821_a_at	0.276
2810410M20Rik (Dpy30)	protein dpy-30 homolog			
Spast	spastin isoform 1		1443074_at	0.452[#]
Slc30a6	zinc transporter 6		1424241_at	0.047
Nlrc4	NLR family CARD domain-containing protein 4			
Yipf4	protein YIPF4		1426417_at	0.381
Birc6	baculoviral IAP repeat-containing 6		1437789_at	0.390
Ttc27	tetratricopeptide repeat protein 27	MRS11814684		

Indicated is the mouse gene symbol, the description, the presence of non-synonymous SNPs, the probe set identifier on the affymetrix (UCHSC BXD Whole Brain M430 2.0 November 2006 RMA, Accession number: GN123) with the highest Pearson correlation and its correlation coefficient. [#]Correlation between expression in brain and the percentage of PPI is significant ($P < 0.05$), but does not withstand correction for multiple testing (74 correlations tested between 74 probe sets targeting the 33 genes and PPI score, Bonferroni corrected $P < 6.67 * 10^{-4}$).

Supplementary Table 2 | Genes located within the confidence interval of response variability QTL on chromosome 16.

GeneSymbol	Description	Probe set with highest correlation	Pearson R (n = 22)
A930003A15Rik	Mus musculus adult retina cDNA, RIKEN full-length enriched library, hypothetical protein, full insert sequence.		
Klhl6	kelch-like protein 6	1437886_at	0.082
Klhl24	kelch-like protein 24	1429351_at	-0.109
Yeats2	YEATS domain-containing protein 2 isoform 3		
Map6d1	MAP6 domain-containing protein 1	1433630_at	-0.041
Parl	presenilins-associated rhomboid-like protein,	1433478_at	0.072
Cyp2ab1	cytochrome P450, family 2, subfamily ab,		
Abcc5	multidrug resistance-associated protein 5	1435683_a_at	0.444[#]
Eif2b5	translation initiation factor eIF-2B subunit	1433886_at	0.066
Dvl3	segment polarity protein dishevelled homolog	1442006_at	-0.278
Ap2m1	AP-2 complex subunit mu	1450894_a_at	-0.215
Abcf3	ATP-binding cassette sub-family F member 3	1426748_s_at	-0.252
BC052055 (Vwa5b2)	von Willebrand factor A domain-containing	1443369_at	-0.018
EG328644 (Vwa5b2)	predicted gene, EG328644		
Alg3	dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl	1460742_at	0.292
Ece2	endothelin-converting enzyme 2 isoform b	1424561_at	0.096
Camk2n2	calcium/calmodulin-dependent protein kinase II	1429204_at	0.069
Ece2	endothelin-converting enzyme 2 isoform d	1424561_at	0.096
Psmd2	26S proteasome non-ATPase regulatory subunit 2	1415831_at	-0.087
Eif4g1	eukaryotic translation initiation factor 4 gamma	1427036_a_at	-0.272
2900046G09Rik (Fam131a)	hypothetical protein LOC78408 precursor; family with sequence similarity 131, member A	1426729_at	-0.115
Clcn2	chloride channel 2	1449248_at	0.078
Polr2h	DNA-directed RNA polymerases I, II, and III	1424473_at	-0.05
Thpo	thrombopoietin precursor	1449569_at	0.269
Chrd	chordin precursor	1417304_at	0.068
2310042E22Rik	hypothetical protein LOC66561	1423494_at	0.443[#]
Ephb3	ephrin type-B receptor 3 precursor	1451550_at	0.335
A830060N17	Mus musculus 10 days neonate cortex cDNA, RIKEN full-length enriched library, unclassifiable		
Vps8	vacuolar protein sorting-associated protein 8	1445796_at	0.125
mKIAA0804 (Vsp8)	Mus musculus 10 days neonate skin cDNA, RIKEN full-length enriched library, clone:4732480N14 product: CDNA FLJ12883 FIS, CLONE NT2RP2003981		

Supplementary Table 2. (*Continued*)

2510009E07Rik	hypothetical protein LOC72190	1428097_at	-0.117
Ehhadh	peroxisomal bifunctional enzyme	1448382_at	0.260
1300002E11Rik	Mus musculus adult male liver cDNA, RIKEN full-length enriched library, clone:1300002E11 product:unclassifiable, full insert sequence.		
AK041461	Mus musculus 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A630012F06 product:unclassifiable, full insert sequence.		
Map3k13	mitogen-activated protein kinase kinase kinase		
Tmem41a	Mus musculus 10 day old male pancreas cDNA, RIKEN full-length enriched library, clone:1810033N04 product:hypothetical Y71A12C.2 CG8408 D2013.10 5730578N08RIK T07F10.4 POSSIBLE containing protein, full insert sequence.	1424191_a_at	-0.016
Liph	lipase member H isoform 1	1456619_at	0.357
Senp2	sentrin-specific protease 2	1451717_s_at	-0.288
Smt3ip2	sentrin-specific protease 2		
Igf2bp2	insulin-like growth factor 2 mRNA-binding		
Sfrs10	transformer-2 protein homolog beta	1419543_a_at	-0.220
Etv5	ETS translocation variant 5	1420998_at	-0.100
Dgkg	diacylglycerol kinase gamma	1445910_at	0.272
9230117E06Rik	Mus musculus adult male epididymis cDNA, RIKEN full-length enriched library, clone:9230117E06 product:unclassifiable, full insert sequence.		
Crygs	beta-crystallin S	1420453_at	-0.245
Tbccd1	TBCC domain-containing protein 1	1436752_at	0.077
Dnajb11	dnaJ homolog subfamily B member 11 precursor	1423151_at	-0.099
AK149453	Mus musculus adult male liver tumor cDNA, RIKEN full-length enriched library, clone:C730014P15 product:unclassifiable, full insert sequence.		
Ahsg	alpha-2-HS-glycoprotein precursor	1455093_a_at	-0.239
Fetub	fetuin-B isoform 3	1449555_a_at	0.428[#]
Hrg	histidine-rich glycoprotein	1449242_s_at	0.097
Kng2	kininogen 2 isoform 2		
Kng1	kininogen-1 isoform 2	1426045_at	0.165
Eif4a2	eukaryotic initiation factor 4A-II isoform a	1450934_at	-0.043
Rfc4	replication factor C subunit 4	1424321_at	-0.136
Adipoq	adiponectin	1447582_x_at	0.389
BC098222	Mus musculus 4 days neonate thymus cDNA, RIKEN full-length enriched library, clone:B630019A10 product:hypothetical protein, full insert sequence.		
C730014E05Rik	Mus musculus adult male liver tumor cDNA, RIKEN full-length enriched library, clone:C730040G15 product:unclassifiable, full insert sequence.		
B630019A10Rik	SubName: Full=BC106179 protein; SubName: Full=Putative uncharacterized protein;		

Supplementary Table 2. (*Continued*)

St6gal1	beta-galactoside alpha-2,6-sialyltransferase 1	1420927_at	0.404
Rtp1	receptor-transporting protein 1		
Masp1	mannan-binding lectin serine protease 1	1438602_s_at	0.220
Masp3	mannan-binding lectin serine protease 1		
AK144897	Mus musculus lung RCB-0558 LLC cDNA, RIKEN full-length enriched library, clone:G730049K06 product:unclassifiable, full insert sequence.		
Rtp4	receptor-transporting protein 4	1418580_at	0.246
Sst	somatostatin precursor	1417954_at	-0.076
Rtp2	receptor-transporting protein 2		
Bcl6	B-cell lymphoma 6 protein homolog	1450381_a_at	-0.223
1110054M08Rik	Mus musculus 18-day embryo whole body cDNA, RIKEN full-length enriched library, clone:1110054M08 product:unclassifiable, full insert sequence.		
Lpp	lipoma-preferred partner homolog isoform 1	1436714_at	0.345
A230028O05Rik	Mus musculus adult male hypothalamus cDNA, RIKEN full-length enriched library, clone:A230028O05 product:unclassifiable, full insert sequence.		
5430420C16Rik (Tprg)	Tprg transformation related protein 63 regulated	1430114_at	0.147
Tp73l	tumor protein p63 isoform		
Trp63	transformation related protein 63	1418158_at	0.354
p73H (Tcp1)	tumor protein p63 isoform		
Leprel1	prolyl 3-hydroxylase 2 precursor	1442486_at	0.184
Cldn1	claudin-1	1437932_a_at	0.337
Cldn16	claudin-16	1420434_at	0.483[#]
Tmem207	transmembrane protein 207		

Indicated is the mouse genesymbol, the description, the probe set (Virginia Commonwealth University, BXD Prefrontal Cortex Saline Control M430 2.0 (Dec06) RMA Dataset, Accession number: GN135) with the highest Pearson correlation and its correlation coefficient. [#]Correlation between expression in prefrontal cortex and impulsive action is significant ($0.01 < P < 0.05$), but does not withstand correction for multiple testing (146 correlations tested between 146 probesets targeting the 79 genes and PPI score, Bonferroni corrected $P < 3.4 * 10^{-4}$). None of the genes harbors non-synonymous SNPs.