Genetic architecture and behavioral analysis of attention and impulsivity

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Chapter 3

Inhibitory control and response latency differences between C57BL/6J and DBA/2J mice in a Go/No-Go and 5-choice serial reaction time task and strain-specific responsivity to amphetamine

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Abstract

Among the best-replicated and most heritable endophenotypes of attention deficit/ hyperactivity disorder (ADHD) are deficits in attention, inhibitory response control and larger intra-individual variability in response latencies. Here, we explored the presence of these heritable ADHD endophenotypes in two commonly used inbred mouse strains, C57BL/6J and DBA/2J, and investigated whether treatment with the stimulant amphetamine affected these phenotypes. Both in an operant Go/No-Go task and 5-choice serial reaction time (5-CSRT) task, DBA/2J mice showed reduced inhibitory response control compared with C57BL/6J mice. Mean correct response latencies of DBA/2J mice were slower in both tasks. Analysis of the distribution of correct response latencies suggested similar processing speed, but DBA/2J mice displayed larger intra-individual variability. Amphetamine did not affect inhibition in the Go/No-Go task but increased omission errors. In contrast, in the 5-CSRTT, amphetamine did not affect omission errors but impaired inhibitory response control, specifically in C57BL/6J mice. The dopamine uptake inhibitor, GBR 12909, mimicked this effect and decreased accurate choice, specifically in C57BL/6J mice, indicating that dopamine modulates inhibitory response control and attention in the murine 5-CSRTT. Amphetamine did not affect response distributions in either task. Furthermore, we extended previous reports on differences in the brain dopamine system of DBA/2J and C57BL/6J mice, by showing differential gene expression levels of three dopamine receptors (Drd1, Drd4 and Drd5) in the mPFC. In conclusion, genetic differences between DBA/2J and C57BL/6J mice translate into multiple ADHD-related phenotypes, indicating that these strains are valuable resources to understand genetic mechanisms underlying ADHD-relevant phenotypes.

Introduction

Attention-deficit/ hyperactivity disorder (ADHD) is a highly heritable psychiatric disorder (Faraone & Biederman, 1998) with a complex genetic architecture. Current theories suggest that multiple neuropsychological deficits can lead to ADHD, such as mild executive dysfunction and/or increased aversion to delay of reinforcement (Castellanos *et al.*, 2006; Solanto *et al.*, 2001). In order to facilitate studies of this complex genetic architecture and neuropsychological heterogeneity, the use of endophenotypes has been proposed (Gottesman & Gould, 2003) that may be more proximal to the genes involved in ADHD. Among the best-replicated (Castellanos & Tannock, 2002) and most promising ADHD endophenotypes for genetic studies (Bidwell *et al.*, 2007) are various executive functions, such as attentional processing and inhibitory response control, and possibly measures of inattention such as intra-individual variability in response latencies. Stimulant treatment (i.e. methylphenidate and amphetamine) improves attentional processing and inhibitory response control,

and decreases intra-individual variability in response speed in ADHD patients (Pietrzak *et al.*, 2006; Spencer *et al.*, 2009), underscoring the clinical relevance of these endophenotypes. Furthermore, the beneficial effect of stimulant treatment is in line with the hypothesis of a dopaminergic dysfunction in ADHD.
Heritable endophenotypes play a pivotal role in the translation of complex human disorders into genetically valid animal models of the disorder, and *vice versa*. Among other genetic animal models (Sagvolden *et al.*, 2005), the comparison of C57BL/6J versus DBA/2J mouse strains seems promising in this respect. These commonly used inbred strains of mice show differences in sustained visual attention and inhibitory response control (Loos *et al.*, 2009; Patel *et al.*, 2006; Pattij *et al.*, 2007; Young *et al.*, 2009a) and increased aversion to delay of reinforcement (Helms *et al.*, 2006). Furthermore, mice of these strains show profound differences in their brain dopaminergic system (Cabib *et al.*, 2002; D'Este *et al.*, 2007) that may be relevant for research on dopaminergic dysfunction in ADHD. In further support of selecting these two inbred strains, the existing panel of recombinant inbred strains derived from crossbreeding C57BL/6J × DBA/2J mice (BXD; Peirce *et al.*, 2004) potentially provides the opportunity to genetically dissect ADHD-relevant phenotypes and identify genetic loci and/or genes that contribute to these phenotypes.
In the present study, we investigated putative ADHD-related phenotypes in C57BL/6J and DBA/2J mice, namely inhibitory response control, visuospatial attention, and intra-individual variability in response latencies. These measures were assessed in an operant Go/No-Go and 5-choice serial reaction time (5-CSRT) task. Although both tasks measure inhibitory control, the inhibition of commission errors in the Go/No-Go task and the inhibition of premature responses in the 5-CSRTT may not require identical control mechanisms (Eagle & Baunez, 2010). Therefore these tasks might act complementary to describe putative differences in inhibitory control between C57BL/6J and DBA/2J mice.
The interest in intra-individual variability in response latencies in human studies has grown over the last decade (e.g. Bidwell *et al.*, 2007; Castellanos *et al.*, 2006; Castellanos & Tannock, 2002; Klein *et al.*, 2006; Spencer *et al.*, 2009) following the observation of increased intra-individual variability in ADHD (Leth-Steensen *et al.*, 2000; Sergeant & van der Meere, 1990). Whereas the peak of a response latency distribution could be viewed as an index of processing speed, larger intra-individual variability has been interpreted as lapses in attention (Leth-Steensen *et al.*, 2000; Sabol *et al.*, 2003). In the present study, we employed the number of correct response latencies in both the Go/No-Go tasks and 5-CSRTTs to analyze response distributions and to investigate potential strain differences in processing speed and lapses in attention.
Stimulant treatment modulates inhibitory control (Eagle *et al.*, 2008; Pietrzak *et al.*, 2006), primarily via dopaminergic mechanisms (Pattij & Vanderschuren, 2008), and decreases intra-individual variability in response latencies in humans and rats (Pietrzak *et al.*, 2006; Sabol *et al.*, 2003; Spencer *et al.*, 2009). To

investigate dopaminergic modulation of these cognitive processes and possible translational validity, we here investigated the effects of amphetamine in the murine Go/No-Go and 5-CSRTT. Because of the aforementioned differences in the brain monoamine systems between C57BL/6J and DBA/2J mice, we anticipated that these strains could respond in a strain-specific manner.
Finally, several genetic association studies implicated dopamine receptors in the etiology of ADHD (Faraone *et al.*, 2001; Lowe *et al.*, 2004; Misener *et al.*, 2004) and interfering with these receptors in the medial prefrontal cortex (mPFC) is known to affect various executive functions, such as attention, correct response time and working memory (Granon *et al.*, 2000; Sawaguchi & Goldman-Rakic, 1991). Therefore, to explore putative differences between C57BL/6J and DBA/2J in dopamine receptor signaling that might underlie observed behavioral differences, we compared gene expression levels of dopamine receptors in the medial prefrontal cortex (mPFC).

Materials and Methods

Subjects and housing
Male seven-week-old mice were obtained from Charles River (Go/No-Go task: n = 12 C57BL/6J and n = 11 DBA/2J mice; gene expression measurement: n = 16 C57BL/6J and n = 16 DBA/2J mice) or our own breeding colony at Harlan Germany (5-CSRT: n = 16 C57BL/6J and n = 10 DBA/2J mice). 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. Mice subjected to behavioral testing were food-restricted to gradually decrease their body weight to 90% of the initial body weight the following 7 days. Mice were placed in the operant cages for 5 days each week for testing.

Operant chambers
Operant chambers (MEDNPW-5M, Med Associates Inc., St Albans, VT, USA) were equipped with five response holes, a food magazine with a reward dispenser (12 mg pellet, Formula P; Research Diets Inc., New Brunswick, NJ, USA) at the opposite wall, a house light, a 4500 Hz tone generator (Mallory Sonalert Products Inc., Indianapolis, IN, USA), and placed in sound-attenuating ventilated cubicles. Both response holes and the food magazine contained yellow LED stimulus lights and infrared response detectors.

Drug injections
(+)–Amphetamine sulfate (O.P.G., Utrecht, The Netherlands) and GBR 12909 (Sigma, St. Louis, MO, USA) were dissolved in sterile saline (0.9 g/l NaCl) and aliquots were stored at –20ºC. Drugs were injected intraperitoneally in a volume

of 10 ml/kg body weight, 10 min before testing. Different doses of the same drug (Go/No-Go task: amphetamine 0.5 and 1.0 mg/kg; 5-CSRTT: amphetamine 0.25, 0.5 and 1.0 mg/kg and GBR12909 2.5, 5 and 10 mg/kg) and vehicle were administered in a Latin square design on Tuesday and Fridays, resulting in at least two wash out days in between each dose. The dose ranges were chosen based on the reported effectiveness in operant procedures taxing impulsivity (Isles *et al.*, 2003; van Gaalen *et al.*, 2006a).

Go/No-Go task

In this paradigm, the cue light in the middle of the five response holes (i.e. hole 3) was designated as the "start stimulus" and the cue light in the response hole immediately to the left or right (i.e. hole 2 or 4, counterbalanced across mice and strains) was designated as the "Go stimulus". All sessions ended if a subject completed 100 trials or after 30 min, whichever came first.

Shaping of Go-response step 1. In the first session, both the start stimulus and Go stimulus were switched on, and after a response into one of these two holes a reward was delivered and both cue lights were turned off. Subsequently, following an inter-trial interval (ITI) of 10 s both the start stimulus and Go stimulus were switched on. After 7 sessions all animals completed more than 40 trials in 30 min.

Shaping of Go-response step 2. In the next 5 sessions, the start of a trial was signaled by the illumination of the start stimulus. A response in this hole switched off the start stimulus and was immediately followed by the presentation of the Go stimulus. A response into the Go stimulus hole resulted in the delivery of a reward, turned off the stimulus light and after an ITI of 10 s the next trial started. Premature Go-responses in an inactive Go stimulus hole resulted in a 5 s time out (TO) during which both house light and stimulus lights were turned off.

Shaping of Go-response step 3. In the subsequent 9 sessions, responding at a VR3 schedule in the illuminated stimulus-hole was required to initiate the Go stimulus. The latency between the onset of the Go stimulus and a response into the Go stimulus hole was recorded for each trial and reflected the Go-response latency (GoRT).

Shaping of Go-response step 4. In the next 10 sessions, the Go stimulus was only switched on for the duration of an individually-titrated Limited Hold period (LH). A Go-response during the LH time resulted in the delivery of a reward. An omission of a Go-response during the LH time was followed by a 5 s time out, during which both house light and stimulus lights were turned off. In the first five sessions, the GoRTs of the previous session for each mouse were ranked, and the LH time was set to the 90^{th} percentile of the GoRTs of the previous session. In the last 5 sessions, the LH times were manually titrated for each mouse individually such that in 30% of Go trials the mouse did not have a chance to respond within the LH time (30% of Go omissions).

Go/No-Go task. In the final Go/No-Go paradigm, an auditory No-Go signal (4500 Hz) was presented randomly in 20% of the Go trials. The No-Go-signal was switched on and off simultaneous with the illumination of the cue light in the Go-stimulus hole for the duration of the LH period. A response (Go) during a No-Go trial resulted in a TO, whereas inhibiting a response during these trials resulted in the delivery of a reward. For two sessions, the LH during No-Go trials was set to the individual mean GoRT to facilitate the acquisition of inhibition. Subsequently, during the first until the third Go/No-Go session, the Go and No-Go LH were both set to the individual titrated level as assessed during the last session of shaping step 4. Several mice of the DBA/2J strain showed difficulties in learning to inhibit responding during No-Go trials. Therefore, all DBA/2J mice received 3 additional Go/No-Go training sessions. Subsequently, 7 badly performing DBA/2J mice ($P_{inhibition} < 0.1$; see below) received 2 additional Go/No-Go training sessions during which the auditory No-Go signal was presented randomly in 33 and 50% of Go-trials. Mice were trained for 15 standard Go/No-Go sessions, during which their individual LH was again titrated such that the number of omissions during Go trials was 30% to force mice to respond as quickly as possible. The average performance over the two following sessions was taken as baseline performance. In this task, inhibitory control ($P_{inhibition}$) was calculated, similar to previous studies [3, 32], from $P_{No\text{-}Go}$, (number of omitted No-Go trials / number No-Go trials) by taking $P_{omission}$ (number of omitted Go trials / number of Go trials) into account: $P_{inhibition} = [(P_{No\text{-}Go} - P_{omission}) / (1 - P_{omission})]$. When the percentage of omissions during Go-trials was 100 % ($P_{omission} = 1$; observed only after drug infusions), $P_{inhibition}$ was set to 1.

5-CSRTT

Mice were trained on a individually-paced schedule to perform the 5-CSRTT, as described previously (Loos *et al.*, 2009). Mice were habituated to the operant cages for one session of 20 min, during which the house light was switched off, and the stimulus lights in the magazine and stimulus holes turned off. In the subsequent 4 sessions, rewards were distributed into the magazine at variable intervals (ITI; 4, 8, 16 or 32 s), which coincided with switching on the magazine stimulus light. An ITI was only initiated when the previous reward had been collected as indicated by a magazine response, after which the magazine stimulus light was off. A session ended after 25 min or sooner when the criterion of 50 rewards was reached. In the next sessions, a trial started by the illumination of all five stimulus holes. An instrumental response into any of these holes switched off the light in all five stimulus holes, switched on the stimulus light in the magazine and delivered reward into the magazine. Sessions lasted for 25 min or 60 earned rewards. As soon as mice earned 50 or more rewards in two sessions they commenced the next training phase, or after 17 sessions. Subsequent trials started by the illumination of only one stimulus hole. Responses into the non-illuminated holes were without consequence. As soon as mice earned 50 or more

rewards in two sessions they commenced to the next training phase, or after 10 sessions. Finally, mice reached 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 LH 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 omissions was defined as [100 × (omissions) / (omissions + number of correct and incorrect responses)]. Accurate choice or accuracy, the commonly used index of visuospatial attention in the 5-CSRTT, was defined as [100 × (number of correct responses) / (number of correct and incorrect responses)]. Inhibitory response control in this task, in terms of the percentage of 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 if the subject reached criterion performance (omissions < 30%, accuracy > 60%, started trials > 50) or after 10 sessions. Baseline 5-CSRTT performance was calculated from the 6th until 10th session at SD of one second.

Intra-individual variability in response latencies in Go/No-Go and 5-CSRTT
Several methods have been proposed to derive indices of response speed and intra-individual variability from the distribution of response latencies (Leth-Steensen *et al.*, 2000; Sabol *et al.*, 2003) that converge in the assumption that the peak of the distribution measures sensory-motor processing speed while the tail (positive skew) of the distribution measures intra-individual variability, also known as lapses in attention (Leth-Steensen *et al.*, 2000). Both for Go-responses in the Go/NoGo task and correct responses in the 5-CSRTT we used the mode of the response latencies as a measure of sensory-motor processing speed. The mode was calculated according to the half rang method (HRM; Hedges & Shah, 2003). HRM uses the densest half of samples in an iterative fashion to estimate the mode. The difference between the mode and the mean (devmode) of the response latencies was used as a measure of the intra-individual variability.

Gene expression measurements
An independent set of mice (4 pools of mice, n = 8 per pool) that had not been subjected to food restriction was used for real time quantitative PCR

measurements (RT-qPCR). The mPFC was freshly dissected on ice and frozen (–80° C) until further use. RNA was isolated (Loos *et al.*, 2009), DNAse-treated and reverse transcribed to cDNA using random hexanucleotide primers. Gene expression levels of dopamine receptor 1 through 5 (Drd1 – Drd5) were analyzed (ABI Prism® SDS 7900, Applied Biosystems Inc., Foster City, CA, USA) using a SYBR Green approach (cDNA equivalent of 40 ng RNA in a total volume of 10 µl) with 300 nM gene specific primers. Expression levels were normalized to the geometric mean of 2 housekeeping genes (β-actin and HPRT).

Statistical analysis

Differences between strains were analyzed using one-way analysis of variance (ANOVA), the effects of treatment or session were evaluated using repeated measures ANOVA with strain as between-subjects variable (in case of drug administration) and treatment as within-subjects variable. When Mauchly's test for sphericity of data was significant, more conservative Huynh–Feldt corrected degrees of freedom and subsequent probability values were reported. In case of a statistically significant effect of treatment, paired-sample t-tests were used to assess task manipulation or dose effects within strains. In case of statistically significant strain × treatment interaction effects, independent sample t-tests were used to evaluate the differences between strains. All data are depicted as means +/- standard error of the mean (SEM), and the level of significance was set at $P < 0.05$. All analyses were performed using the Statistical Package for the Social Sciences for Windows version 15.0 (SPSS, Chicago, IL, USA).

Results

Go/No-Go task

Acquisition and baseline responding. The limited hold period was individually titrated such that each mouse omitted around 30% of the Go trials (shaping step 4). By the 10th session, the limited hold was titrated such that the number of omissions of Go stimuli did not differ between strains ($F(1,21) = 1.56$, non-significant (n.s.)). DBA/2J mice required longer limited hold times ($F(1,21) = 23.29$, $P < 0.001$) compared with C57BL/6J mice. After introduction of the No-Go signal, from the 1st until the 3rd Go/No-Go session, DBA/2J mice consistently showed a lower $P_{inhibition}$ than C57BL/6J mice (strain: $F(1,21) = 36.49$, $P < 0.001$) without an interaction with session number (session × strain: $F(2,42) = 0.043$, n.s.). By the third session (**Fig. 1a**), 92% of the C57BL/6J mice (11 out of 12) showed inhibition of Go-responses during No-Go trials ($P_{inhibition} > 0$) whereas only 36% (7 out of 11) of the DBA/2J mice did. Even when only mice with $P_{inhibition} > 0$ were evaluated, C57BL/6J mice showed a higher $P_{inhibition}$ than DBA/2J mice (strain: $F(1,13) = 4.85$, $P < 0.05$) (**Fig. 1a**).

After prolonged training in the Go/No-Go task, all C57BL/6J mice (12 out 12) and ten DBA/2J mice (10 out of 11) consistently showed a higher percentage of inhibition during No-Go trials than omissions during Go trials (i.e. $P_{inhibition} > 0$). Similar to the first three sessions, DBA/2J mice displayed a significant lower $P_{inhibition}$ than C57BL/6J mice ($F(1,21) = 5.00$, $P < 0.05$). Because the lack of inhibition during No-Go trials can be the result of poor inhibitory control, or failure to properly acquire the Go/No-Go task, only mice with $P_{inhibition} > 0$ were considered for analyses of baseline performance and the effects of amphetamine. Even under these conditions, a trend towards a lower $P_{inhibition}$ in DBA/2J mice was observed compared with C57BL/6J mice ($F(1,20) = 4.06$, $P = 0.057$) in the remaining mice (**Fig. 1a**). During the prolonged training phase the LH times were titrated for each individual mouse to reach a 30% omission rate during Go-trials. Indeed, strains did not differ in their omission rate of Go trials ($F(1,20) = 2.60$, n.s.), but significantly differed in the required LH to achieve this level of performance ($F(1,20) = 41.91$, $P < 0.001$).
Furthermore, C57BL/6J mice and DBA/2J mice differed significantly in their mean GoRT ($F(1,20) = 6.40$, $P < 0.05$) and LH ($F(1,20) = 41.90$, $P < 0.001$).

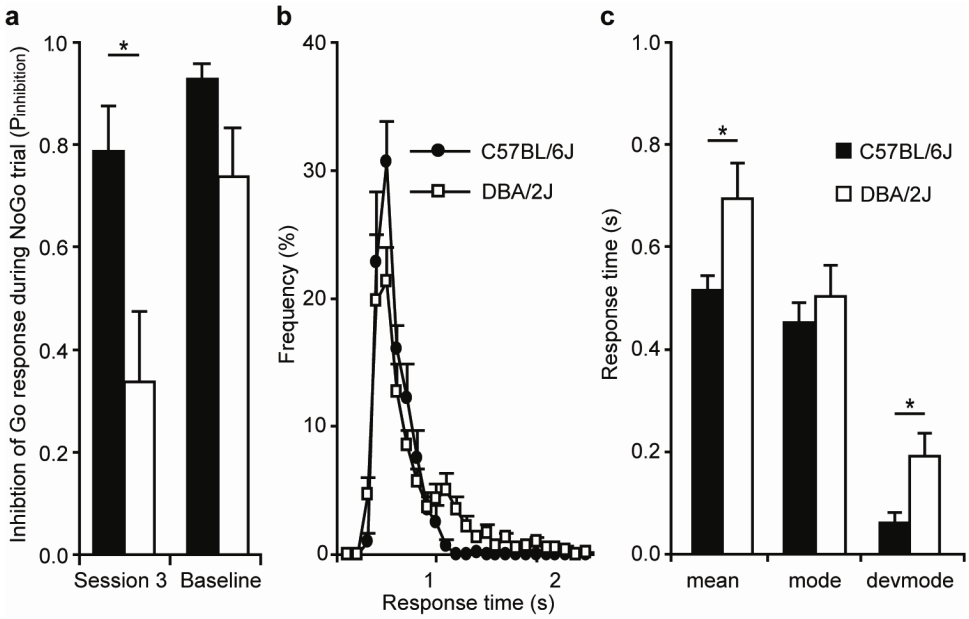

Figure 1 | Baseline Go/No-Go task performance. (**a**) During the third Go/No-Go session (session 3), DBA/2J mice showed impaired inhibitory response control compared with C57BL/6J mice. Only mice were included that showed a $P_{inhibition} > 0$, as indicated by numbers above the bars. After prolonged training (Baseline), most mice successfully acquired the task and a trend toward lower inhibitory response control in DBA/2J mice was observed. (**b**) Distribution of GoRTs for each strain (bin size 0.1 s). The frequency was calculated for each mouse, by dividing the number of correct responses within a time bin by the total number of correct responses of the respective mouse. (**c**) Representation of the mean GoRT, the mode of the Go-response distribution and the intra-individual variability in Go-response latencies (i.e. devmode). * $P < 0.05$.

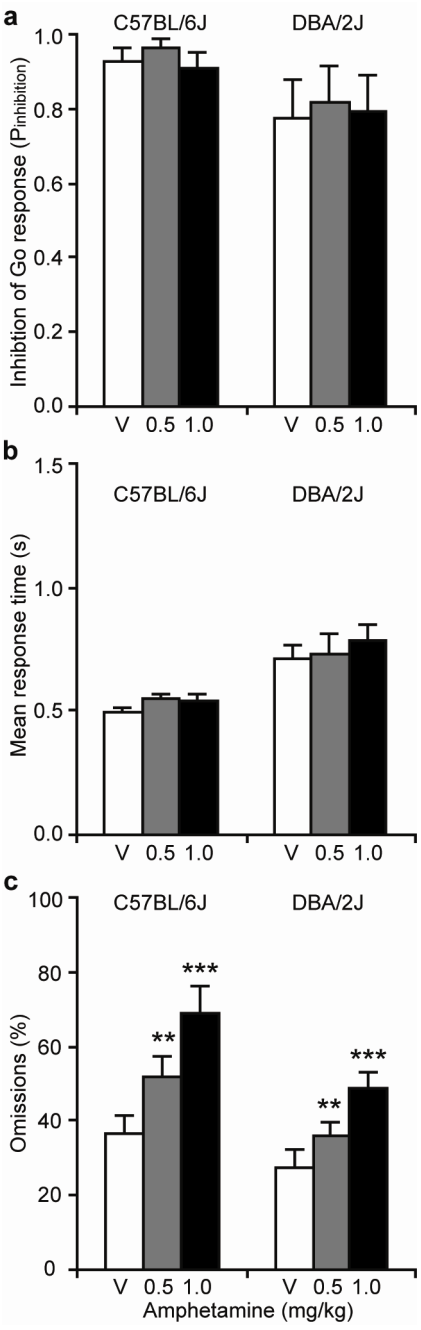

Figure 2 | Effects of amphetamine on Go/No-Go task performance. No effects of different doses of amphetamine (0.5 and 1 mg/kg) were observed on (**a**) inhibitory response control and (**b**) mean GoRTs between strains. (**c**) Amphetamine significantly increased omissions of Go-responses. ** $P < 0.01$, *** $P < 0.001$, V = Vehicle.

DBA/2J mice showed a significantly larger devmode ($F(1,20) = 8.92$, $P < 0.01$) with no significant difference in mode ($F(1,20) = 0.50$, n.s.) (**Fig. 1b-c**), suggesting that the difference in mean GoRT was likely due to a larger intra-individual variability in GoRTs in DBA/2J mice. The two strains did not differ in number of premature Go-responses ($F(1,20) = 0.40$, n.s.).

Effects of amphetamine on Go/No-Go performance. At the highest dose, one C57BL/6J mouse omitted to respond during all Go trials and was therefore excluded from further analyses. Amphetamine (**Fig. 2**) did not significantly affect $P_{inhibition}$ (dose: $F(2,40) = 1.15$, ns; dose × strain: $F(2,40) = 0.17$, ns; strain: $F(1,20) = 2.27$, n.s.) (**Fig. 2a**), number of premature Go-responses (dose: $F(2,40) = 1.41$, ns; dose × strain: $F(2,40) = 0.91$; strain: $F(1,20) = 0.60$, n.s.), mean GoRT (dose: $F(2,38) = 0.47$, ns; dose × strain: $F(2,38) = 1.47$, ns; strain: $F(1,19) = 18.05$, $P < 0.001$) (**Fig. 2b**), mode of GoRTs (dose: $F(1.70,33.89) = 0.18$, ns; dose × strain: $F(1.70,33.89) = 1.75$, ns; strain: $F(1,20) = 5.18$, $P < 0.05$) or intra-individual deviation from the mode (dose: $F(2,38) = 0.83$, ns; dose × strain: $F(2,38) = 0.72$, ns; strain: $F(1,19) = 13.26$, $P < 0.01$). In contrast, amphetamine significantly increased the omission rate during Go trials (**Fig. 2c**; dose: $F(2,40) = 40.28$, $P < 0.001$) in both strains (dose × strain interaction: $F(2,40) = 1.77$, ns; strain: $F(1,20) = 4.71$, $P < 0.05$) and post hoc testing revealed a significant higher percentage of omissions after 0.5 mg/kg ($P < 0.01$) and 1.0 mg/kg ($P < 0.001$).

5-CSRT

Acquisition and baseline responding. All mice completed the standard (Loos *et al.*, 2009) shaping phase including the first five sessions at a baseline SD of 1 s in approximately 40 training days with no difference between strains (strain: $F(1,24) = 1.94$, n.s.). In the subsequent 5 sessions, significant strain differences were observed in the percentage of premature responses (**Fig. 3a**; strain: $F(1,24) = 5.08$, $P < 0.05$) and the mean correct response time (strain: $F(1,24) = 9.59$, $P < 0.01$). Analysis of response latencies (**Fig. 3b-c**) suggested that the slower correct response latencies of DBA/2J mice were the result of a different mode of the response latencies ($F(1,24) = 7.47$, $P < 0.05$) and not of intra-individual variability in the deviation of the mode of response latencies ($F(1,24) = 0.16$, n.s.). No strain differences were observed in the number of omissions (strain: $F(1,24) = 0.18$, n.s.) and accurate choice (strain: $F(1,24) = 0.14$, n.s.).

Effects of amphetamine on 5-CSRTT performance. Amphetamine (**Fig. 4**) increased the percentage of premature responses (dose: $F(2.74,60.20) = 3.95$, $P < 0.05$) in a strain-dependent fashion (dose × strain: $F(2.74,60.20) = 3.67$, $P < 0.05$; strain: $F(1,22) = 1.27$, n.s.). Post hoc testing showed no significant effect in

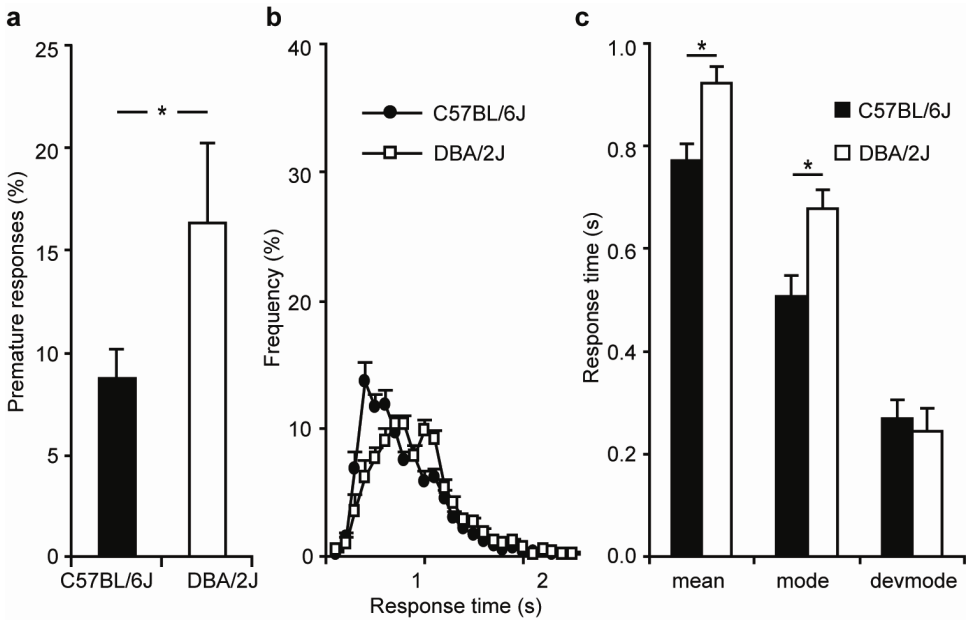

Figure 3 | Baseline 5-CSRTT performance. (**a**) DBA/2J mice made more premature responses than C57BL/6J mice. (**b**) Distribution of GoRTs for each strain (bin size 0.1 s). The frequency was calculated for each mouse, by dividing the number of correct responses within a time bin by the total number of correct responses of the respective mouse. (**c**) Representation of the mean correct response time, the mode of the correct response distribution and the intra-individual variability in GoRTs (i.e. devmode). * $P < 0.05$.

DBA/2J mice (dose: $F(1.60,11.18) = 2.68$, n.s.), but only in C57BL/6J mice amphetamine increased the percentage of premature responses (dose: $F(1.31,19.66) = 6.20$, $P < 0.05$) at the highest dose compared with saline ($P < 0.05$). The apparent, but non-significant effect of 0.5 mg/kg amphetamine on the percentage of premature responses in DBA/2J mice was due to one individual with 48.7 % premature responses, which did not show any outlying behavior on other parameters during that same session.
Amphetamine did not significantly affect mean correct response time (dose: $F(1.24,27.18) = 0.30$, ns; dose × strain: $F(1.24,27.18) = 0.39$, ns; strain: $F(1,22) = 0.42$, n.s.), mode (dose: $F(1.53,33.65) = 0.45$, ns; dose × strain: $F(1.53,33.65) = 0.68$, ns; strain: $F(1,22) = 0.28$, n.s.), intra-individual variability in the deviation of the mode of the response latencies (dose: $F(3,66) = 0.53$, ns; dose × strain: $F(3,66) = 0.73$, ns; strain: $F(1,22) = 0.05$, n.s.), errors of omission (dose: $F(1.43,31.51) = 2.20$, ns; dose × strain: $F(1.43,31.51) = 0.68$, ns; strain: $F(1,22) = 1.39$, n.s.) or accurate choice (dose: $F(3,66) = 0.13$, ns; dose × strain: $F(3,66) = 0.50$, ns; strain: $F(1,22) = 2.05$, n.s.).

Effects of GBR 12909 on 5-CSRTT performance. The selective dopamine uptake inhibitor GBR 12909 (**Fig. 4**) significantly increased the percentage of premature responses (dose: $F(1.92,44.11) = 5.26$, $P < 0.01$), an effect which was dependent upon strain (dose × strain: $F(1.92,44.11) = 4.38$, $P < 0.05$; strain: $F(1,23) = 1.99$, n.s.). Post hoc testing revealed no significant effect in DBA/2J mice (dose: $F(3,24) = 0.22$, n.s.), whereas in C57BL/6J mice the percentage of premature responses was increased (dose: $F(1.60,24.02) = 11.05$, $P < 0.001$) both after 5 and 10 mg/kg GBR 12909 ($P < 0.05$ and $P < 0.001$, respectively). GBR 12909 did not affect the mean correct response latencies (dose: $F(2.67,61.23) = 0.58$, ns; dose × strain: $F(2.67,61.23) = 1.76$, ns; strain: $F(1,23) = 14.75$, $P < 0.001$), mode (dose: $F(3,69) = 1.41$, ns; dose × strain: $F(3,69) = 0.17$, ns; strain: $F(1,23) = 6.02$, $P < 0.05$), intra-individual deviation from the mode (dose: $F(2.18,50.14) = 1.56$, ns; dose × strain: $F(2.18,50.14) = 0.52$, ns; strain: $F(1,23) = 3.15$, n.s.) and omissions (dose: $F(3,69) = 1.15$, ns; dose × strain: $F(3,96) = 1.07$, ns; strain: $F(1,23) = 4.81$, $P < 0.05$). With respect to accurate choice (**Fig. 4f**), no significant main effect of GBR 12909 was observed (dose: $F(3,69) = 0.88$, n.s.), but an interaction with strain was observed (dose × strain: $F(3,69) = 3.07$, $P < 0.05$; strain: $F(1,23) = 2.91$, n.s.). Post hoc testing revealed a significant decrease in accurate choice in C57BL/6J mice at the highest dose of GBR 12909 compared with saline ($P < 0.05$). A more detailed analysis revealed that the number of incorrect responses increased significantly ($P < 0.05$) from 2.1 ± 0.6 under saline to 4.6 ± 0.8 after 10 mg/kg GBR 12909, whereas the number of correct responses decreased significantly ($P < 0.05$) from 30.1 ± 1.5 to 26.8 ± 1.4.

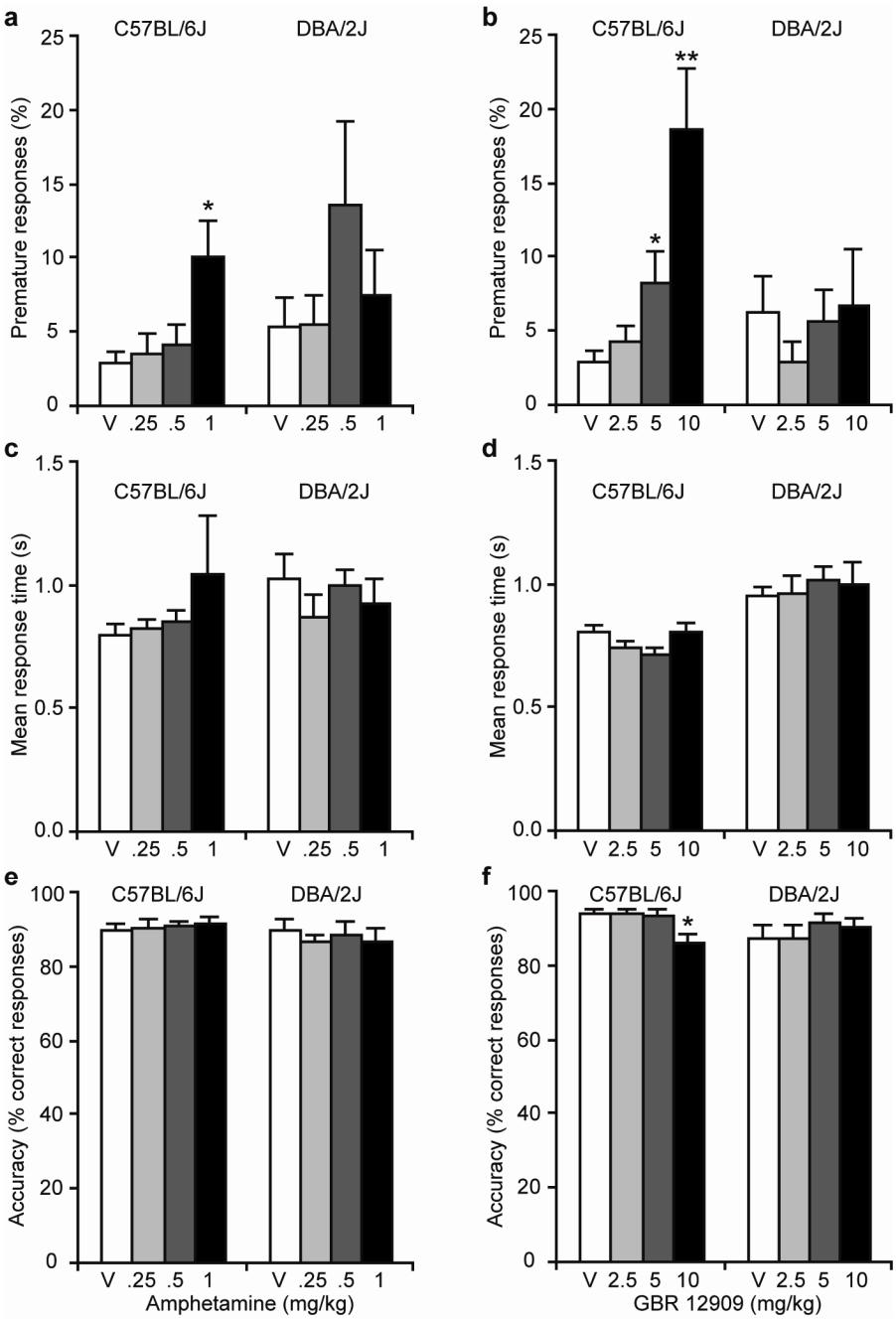

Figure 4 | Effects of amphetamine and GBR 12909 on 5-CSRTT performance. Amphetamine and GBR 12909 (**a**, **b**) increased the percentage of premature (impulsive) responses, but did not affect (**b, e**) the mean correct response time or (**e, f**) the accuracy. * $P < 0.05$, ** $P < 0.01$, V = Vehicle.

mPFC gene expression levels of dopamine receptors

DBA/2J mice showed a significantly higher gene expression level of dopamine D1, D4 and D5 receptors in the mPFC compared with C57BL/6J mice (**Fig. 5**; Drd1: 1.37 fold, $F(1,7) = 8.71$, $P < 0.05$; Drd4: 2.09 fold, $F(1,7) = 17.45$, $P < 0.01$; Drd5: 1.31 fold, $F(1,7) = 8.96$, $P < 0.05$). In addition, dopamine receptor D2 and D3 could not reliably be quantified due to low gene expression levels; more than 35 PCR cycles were required to reach the detection threshold.

Discussion

The present study demonstrates that two commonly used inbred strains of mice differ in inhibitory control, response speed and response variability, which are among the best replicated endophenotypes of ADHD (Castellanos & Tannock, 2002). In line with the earlier suggestion that both DBA/2J mice and C57BL/6J mice harbor ADHD-related phenotypes (Cabib *et al.*, 2002), we observed strain differences in inhibitory control, response latencies and the tail of the response time distribution as measured in two different cognitive paradigms. Furthermore, we showed that inhibitory control can be modulated by amphetamine and GBR12909 in a strain-specific manner. Finally, in addition to previously reported differences in the brain dopaminergic system between these two strains (Cabib *et al.*, 2002; D'Este *et al.*, 2007), we observed a higher gene expression level of Drd1, Drd4 and Drd5 in the mPFC of DBA/2J compared with C57BL/6J mice.

ADHD-related phenotypes in C57BL/6J and DBA/2J mice

One of the principle reasons to select C57BL/6J and DBA/2J strains of mice in the present study is that these strains are the founders of the BXD recombinant inbred strain of mice (Peirce *et al.*, 2004). Employing this panel of BXD strains in search for ADHD-related phenotypes, future studies may be able to identify genetic loci and/or genes that contribute to these phenotypes or specific BXD strains that may actually capture multiple ADHD-related phenotypes within one strain. Thus, in view of this, observing strain differences in various ADHD

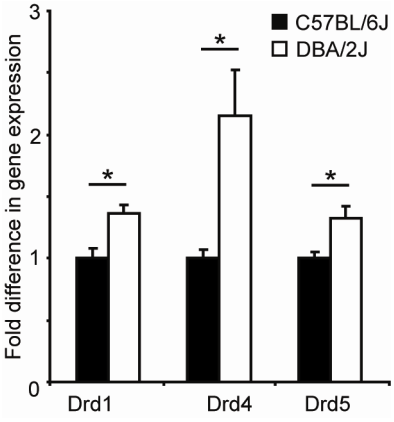

Figure 5 | Gene expression levels for Drd1, Drd4 and Drd5 in DAB/2J and C57BL/6J mice. DBA/2J mice have a significantly higher transcript levels of Drd1, Drd4 and Drd5 in the mPFC compared with C57BL/6J mice, as measured by RT-qPCR. * P < 0.05.

related phenotypes in these founder strains would be a first indicator of the fruitfulness of this approach.

Inhibitory response control – Both in the Go/No-Go and 5-CSRTT DBA/2J mice showed poorer inhibitory response control capacities. DBA/2J mice had a reduced ability to inhibit responding during No-Go trials when an auditory No-Go signal was presented, although this difference was not maintained after prolonged baseline training (**Fig. 1**). This reduced ability to inhibit responding was probably not due to the reported hearing difficulties of DBA/2J mice (Erway *et al.*, 1993), as a pilot study indicated that the auditory signal used in the current study was sufficient to elicit conditioned approach behavior to a food predictive stimulus in this strain (unpublished observations). In the 5-CSRT, reduced inhibitory control of DBA/2J mice was apparent in terms of the ability to withhold premature responses to a food predictive stimulus. Whereas, to our knowledge, this is the first study to compare C57BL/6J and DBA/2J mice in the Go/No-Go task, several previous studies have compared these strains in the 5-CSRTT. In line with these studies and despite differences in equipment and protocols (Loos *et al.*, 2009; Patel *et al.*, 2006; Pattij *et al.*, 2007), DBA/2J mice showed poorer inhibitory control than C57BL/6J mice, indicating that this ADHD-related phenotype is robustly differentiating these strains.

Visuospatial attention – With respect to visuospatial attention that was only assessed in the 5-CSRTT in terms of accuracy of responses both strains showed a similar behavioral performance. In contrast, one previous study reported better performance of DBA/2J mice (Pattij *et al.*, 2007), whereas other studies (Greco *et al.*, 2005; Patel *et al.*, 2006) reported that C57BL/6 mice outperformed DBA/2 mice. It is possible that the short LH of 2 s we employed previously (Pattij *et al.*, 2007) partly explains this discrepancy, as the study by Patel and colleagues (2006) and the current study have used a longer LH period (5 s and 4 s, respectively). In support of this, mean incorrect response latencies of both C57BL/6J (2.0 s) and DBA/2J (2.4 s) mice were much longer than their correct response latencies, and the strain difference for incorrect response latencies nearly reached significance ($P = 0.056$). Hence, the shorter LH in our previous work (Pattij *et al.*, 2007) might have lowered the number of incorrect responses and increased omission rate in particular in DBA/2J mice, thereby resulting in higher accurate choice in this strain.

Correct response latencies and intra-individual variability – Although DBA/2J mice had lower mean correct response latencies in both the Go/No-Go and 5-CSRTT, analyses of the response time distributions indicated task-specific differences. In the Go/No-Go task, the mode of the response latencies (peak of response distribution) task did not differ, suggesting that both strains have similar sensory- and motor-processing times (Leth-Steensen *et al.*, 2000; Sabol *et al.*,

2003). However, the intra-individual variability in correct response latencies (devmode) of DBA/2J mice was significantly larger, suggesting that this strain might have had more lapses in attention towards the Go stimulus. Upon individual analysis, it appeared that this delayed response peak was present in the histogram of the majority of DBA/2J mice and not resulting from a few mice with slower modes. Together, the observations in the Go/No-Go test suggest that sensorimotor processing speed in DBA/2J mice is not different from C57BL/6J mice, but the former strain might display lapses in attention, an important endophenotype of ADHD.
In contrast to the Go/No-Go task, the slower mean response time of DBA/2J mice in the 5-CSRTT appeared to be due to a slower mode of the response latencies in this strain instead of a slower devmode. One critical difference between the Go/No-Go and 5-CSRTT paradigm is the temporal predictability of the presentation of a cue light in the 5-CSRTT paradigm that we employed. Whereas in the Go/No-Go task the Go stimulus was presented randomly after one, two or three responses (variable ratio 2 schedule) in the start-stimulus hole in order to prevent prepotent response patterns, in the 5-CSRTT the visual stimulus onset always occurred 5 s following a magazine response to start a trial. Hence, the significantly slower mode of response latencies of DBA/2J mice in the 5-CSRTT is probably not explained by sensorimotor processing speed, but rather by the reduced ability of DBA/2J mice to use this temporal information to focus their attention to the visual stimulus 5 s later. This notion is further supported by previous studies (Patel *et al.*, 2006; Pattij *et al.*, 2007; Young *et al.*, 2009a) in which mice were either not required to start a trial or the inter-trial interval was unpredictable such that neither of the strains could effectively use a timing strategy. Indeed, in these studies correct response latencies did not differ between C57BL/6J and DBA/2J mice (Patel *et al.*, 2006; Pattij *et al.*, 2007; Young *et al.*, 2009a). Unfortunately, to date, there is no data available on the timing ability of DBA/2J mice to further support this idea. In fact, a study on strain differences measuring timing ability might be worthwhile, as disturbances in timing ability have been linked to ADHD (Toplak *et al.*, 2006).

Pharmacological effects on Go/No-Go and 5-CSRTT performance
The availability of transgenic mouse lines has led to the development of a vast number of operant tasks to assess cognitive function in mice over the last decade. Nonetheless, the pharmacological evaluation of most of these tasks is lacking. For example, to date, there is no study describing pharmacological manipulation of Go/No-Go performance in mice. With respect to the 5-CSRTT, recent studies show that visuospatial attention in mice is enhanced by nicotine (Pattij *et al.*, 2007; Young *et al.*, 2004), in line with the effects in humans and rats (Levin *et al.*, 1998; Levin *et al.*, 1996). In addition, inhibitory response control in mice measured in the same paradigm has recently been demonstrated to be modulated by $GABA_A$, NMDA and serotonin $5\text{-}HT_2$ receptor ligands (Fletcher *et al.*, 2007;

Oliver *et al.*, 2009). Nonetheless, little is known with respect to dopaminergic modulation of visuospatial attention and inhibitory response control in mice. Therefore, in addition to studying phenotypic differences between C57BL/6J and DBA/2J mice in more detail, we evaluated the effects of the stimulant amphetamine in both operant tasks, as the behavioral effects of this compound in rat versions of these tasks largely seem to depend on dopamine transmission (Pattij & Vanderschuren, 2008).

Go/No-Go task – In the current study, amphetamine did not affect $P_{inhibition}$, the principal measure of inhibitory control in our Go/No-Go task. $P_{inhibition}$ is an index of inhibition of commission errors during No-Go trials, corrected for the number of omission errors during Go-trials. Therefore, the lack of effect of amphetamine on $P_{inhibition}$ may be due to the observed robust increase in omission errors during Go trials in both strains. This was not paralleled by an increase in GoRT, suggesting a specific effect of amphetamine on omission errors rather than a general disruption of task performance. With respect to human Go/No-Go studies, stimulants have been found to increase inhibition (Trommer *et al.*, 1991; Vaidya *et al.*, 1998) or, similar to the current results, not affect inhibition (Kratz *et al.*, 2009; van der Meere *et al.*, 1999). However, changes in omission errors have not been reported in human studies. This could be due to a more general procedural difference, as omission errors are scarce in human Go/No-Go studies inhibition (Kratz *et al.*, 2009; van der Meere *et al.*, 1999) in contrast to the current murine version, in which the number of omission errors was titrated to 30% in both strains. Taken together, the unprecedented increase in omission errors in our murine Go/No-Go task after amphetamine administration could be indicative of an enhanced inhibition of Go-responses that was not specific to No-Go trials.
In DBA/2J and C57BL/6J mice, amphetamine did not affect mean GoRTs and intra-individual variability. In human Go/No-Go tasks, stimulants have various effects on these parameters and were found to either decrease (Kratz *et al.*, 2009) or unaltered GoRTs (Vaidya *et al.*, 1998; van der Meere *et al.*, 1999), and moreover, did not affect (Kratz *et al.*, 2009) intra-individual variability. In contrast, results obtained in simple discrimination and Stop Signal paradigms in humans and rats appear less unequivocal, with reports of decreased GoRT and intra-individual variability (Pietrzak *et al.*, 2006; Sabol *et al.*, 2003; Spencer *et al.*, 2009). Together, these findings suggest that the murine Go/No-Go task may not be the most well-suited paradigm to investigate mechanisms by which stimulants affect response time distributions and for this purpose it might be more promising to develop a murine version of the Stop Signal paradigm.

5-CSRTT – In contrast to the Go/No-Go task, we observed that amphetamine increased the number of premature responses in mice in the 5-CSRTT, consistent with findings in rats (Cole & Robbins, 1987; Harrison *et al.*, 1997; van Gaalen *et*

al., 2006a). This effect was only observed in C57BL/6J mice, clearly showing that genotype interacts with drug effects. The lack of effect in DBA/2J mice is not simply explained by an altered metabolism or uptake into the brain, since behavioral effects have been shown with comparable doses in the Go/No-Go task (1.0 mg/kg; present study) and in a conditioned place preference paradigm (Cabib *et al.*, 2000). Importantly, large functional differences of the dopaminergic system of both strains have been reported (Cabib *et al.*, 2002; Puglisi-Allegra & Cabib, 1997; Ventura *et al.*, 2004) that likely affect the behavioral responses to the psychostimulant amphetamine. In further support of this notion we tested the effects of the selective dopamine transporter inhibitor GBR12909 in the 5-CSRTT. Similar to amphetamine, GBR 12909 strongly increased premature responding in C57BL/6J mice suggesting that the effects of amphetamine were dopamine-dependent and mediated by its inhibition of the dopamine transporter rather than norepinephrine or serotonin transporters (Rothman *et al.*, 2001).

Amphetamine and GBR 12909 did not affect mean correct response latencies and intra-individual variability therein in the 5-CSRTT. This is in line with previous studies in rats, in which these compounds did not significantly affect mean response latencies (Cole & Robbins, 1987; van Gaalen *et al.*, 2006a but see Harrison *et al.*, 1997). Intra-individual variability in correct response latencies in this task has not been studied in detail before. In contrast, in a simple discrimination task, amphetamine was found to decrease mean response latencies and intra-individual variability (Sabol *et al.*, 2003). Collectively, similar to our murine version of the Go/No-Go task, stimulant treatment does not clearly affect response time distributions in the 5-CSRTT.

Visuospatial attention was affected by the highest dose of GBR 12909, specifically in the C57BL/6J strain, whereas the doses of amphetamine might have been too low to observe similar effects. This observed decrement in accurate choice may partly be resulting from the increase in premature responses observed at this dose, as these measures have been demonstrated to correlate inversely (Dalley *et al.*, 2008). Tardy premature responses might also be recorded as incorrect responses and thereby lead to a decrease in accurate choice. However, the observed concomitant significant decrease in the total number of correct responses indicates that GBR 12909 did not only decrease inhibitory control, but also visuospatial attention. These data are in line with the effects of GBR 12909 in rats (van Gaalen *et al.*, 2006a) and imply the involvement of dopaminergic neurotransmission in visuospatial attention in mice.

Differences in dopamine receptor gene expression in the mPFC

Given the involvement of the dopamine system in ADHD, we explored the level of expression of three dopamine receptors (Drd1, Drd4 and Drd5) in the mPFC of DBA/2J mice. The higher levels of expression of these receptors extend earlier findings of differences in the mPFC dopaminergic system between these strains

phenotypes in these strains. Furthermore, additional experiments are required to establish a functional relation between dopamine receptor expression and the strain-specific response to amphetamine.

Taken together, this study provides evidence for a generalized reduction in inhibitory control of DBA/2J mice in comparison with C57BL/6J mice that is evident in two tasks measuring aspects of inhibitory control that, according to the present pharmacological data and earlier suggestions, do not depend on identical control mechanisms (Eagle & Baunez, 2010). Most prominently, we showed that these strains also differ in intra-individual variability in response latencies in the Go/No-Go task, suggesting more lapses in attention in DBA/2J mice. Based on these strain differences we anticipate that the panel of BXD recombinant inbred strains, derived from crossbreeding C57BL/6J and DBA/2J (Peirce *et al.*, 2004), will be of use in further developing our understanding of ADHD by genetically dissecting ADHD-relevant phenotypes and identifying the loci/genes involved.
Furthermore, despite the reduction in inhibitory response control and visuospatial attention in the 5-CSRTT by amphetamine and/or GBR 12909, which is opposite to the effect required to treat ADHD, this study clearly indicated that the dopaminergic system modulates these executive functions in the murine version of the 5-CSRTT. From a mechanistic point of view these initial findings warrant further investigation of dopamine transmission in modulating these executive functions in mice. On the other hand, from a clinical point of view, developing other operant paradigms, taxing different aspects of inhibitory control, such as response inhibition in a stop signal paradigm, might be more promising to detect beneficial effects of stimulant treatment (Pattij & Vanderschuren, 2008). Regardless, the strain-specific effects of amphetamine and GBR 12909 clearly indicate that genetic background might influence the response to stimulants. In conclusion, the genetic differences between the two employed inbred strains might contribute to multiple distinguishable ADHD-related phenotypes, suggesting that DBA/2J and C57BL/6J mice and recombinant inbred strains derived thereof likely constitute valuable animal models to study ADHD-related phenotypes.

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