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C57BL/6J MICE EXHIBIT REDUCED DOPAMINE D3 RECEPTOR-MEDIATED LOCOMOTOR-INHIBITORY FUNCTION RELATIVE TO DBA/2J MICE

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Abstract

Previous reports have identified greater sensitivity to the locomotor-stimulating, sensitizing, and reinforcing effects of amphetamine in inbred C57BL/6J mice relative to inbred DBA/2J mice. The dopamine D3 receptor (D3R) plays an inhibitory role in the regulation of rodent locomotor activity, and exerts inhibitory opposition to D1 receptor (D1R)-mediated signaling. Based on these observations, we investigated D3R expression and D3R-mediated locomotor-inhibitory function, as well as D1R binding and D1R-mediated locomotor-stimulating function, in C57BL/6J and DBA/2J mice. C57BL/6J mice exhibited lower D3R binding density (−32%) in the ventral striatum (nucleus accumbens/islands of Calleja), lower D3R mRNA expression (−26%) in the substantia nigra/ventral tegmentum, and greater D3R mRNA expression (+40%) in the hippocampus, relative to DBA/2J mice. There were no strain differences in D3R mRNA expression in the ventral striatum or prefrontal cortex, nor were there differences in D1R binding in the ventral striatum. Behaviorally, C57BL/6J mice were less sensitive to the locomotor-inhibitory effect of the D3R agonist PD128907 (10 µg/kg), and more sensitive to the locomotor-stimulating effects of novelty, amphetamine (1 mg/kg), and the D1R-like agonist $\pm$ -1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8,-diol hydrochloride (SKF38393) (5–20 mg/kg) than DBA/2J mice. While the selective D3R antagonist N-(4-[2,3-dichlorophenyl]-1 piperazinyl)butyl)-2-fluorenylcarboxamide (NGB 2904) (0.01–1.0 mg/kg) augmented novelty-, amphetamine-, and SKF38393-induced locomotor activity in DBA/2J mice, it reduced novelty-induced locomotor activity in C57BL/6J mice. Collectively, these results demonstrate that C57BL/6J mice exhibit less D3R-mediated inhibitory function relative to DBA/2J mice, and suggest that reduced D3R-mediated inhibitory function may contribute to heightened sensitivity to the locomotor-stimulating effects of amphetamine in the C57BL/6J mouse strain. Furthermore, these data demonstrate that comparisons between C57BL/6J and DBA/2J mouse strains provide a model for elucidating the molecular determinants of genetic influence on D3R function.

Keywords

dopamine D1 receptor; PD 128907; NGB 2904; SKF38393; amphetamine; locomotor activity

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Comparative studies of inbred C57BL/6 and DBA/2 mouse strains have revealed molecular, neurochemical, and behavioral differences which suggest a significant influence of genotype on brain mesocorticolimbic dopamine neuro-transmission (*reviewed in* Cabib et al., 2002). Increases in extracellular dopamine levels in response to acute amphetamine (AMPH) treatment are greater in nucleus accumbens (NAc), and lower in medial prefrontal cortex, of C57BL/6 relative to DBA/2 mice (Ventura et al., 2004a,b; Zocchi et al., 1998). Behaviorally, C57BL/6 mice exhibit a greater propensity relative to DBA/2 mice to self-administer or exhibit greater conditioned place preference for drugs of abuse that increase NAc extracellular dopamine levels, including alcohol, morphine, nicotine, cocaine, and AMPH (Carney et al., 1991; Meliska et al., 1995; Orsini et al., 2004, 2005; Seale and Charney, 1991). Neuroadaptations within mesolimbic terminal fields are thought to mediate both propensity to self-administer drugs of abuse (George et al., 1995; Volkow and Li, 2004; Volkow et al., 2004), and psychostimulant-induced locomotor sensitization (Vezina, 2004). It is of interest, therefore, that drug-naïve C57BL/6J mice exhibit greater novelty- and AMPH-induced locomotor activity compared with drug-naïve DBA/2 mice (Cabib, 1993; Cabib et al., 2000; Zocchi et al., 1998), and most (Cabib, 1993; Orsini et al., 2004; Phillips et al., 1994; Tolliver et al., 1994; Tolliver and Carney, 1994) but not all (Koff et al., 1994; Phillips et al., 1998) studies report significant differences in behavioral response to repetitive psychostimulant drug treatment between C57BL/6 and DBA/2 strains. These observations suggest that identification of the molecular mechanisms underlying inbred strain differences in behavioral response to psychostimulant drug treatment may provide a model system for elucidating genetic differences in adaptation of dopamine neurotransmission to drugs of abuse.

Dopamine transmission is mediated via two G protein-coupled dopamine receptor families, D1-like (D1 and D5) and D2-like (D2, D3, and D4) (*reviewed in* Missale et al., 1998). D1R binding ($[^3\text{H}]\text{SCH23390}$) has been found to be similar (NAc and caudate-putamen) (Erwin et al., 1993; Cabib et al., 1998) or greater (striatum) (Ng et al., 1994) in C57BL/6 relative to DBA/2 mice. Strain differences in D2R-like binding have also been reported. D2R-like receptor binding and mRNA expression are greater in the striatum of C57BL/6 relative to DBA/2 mice (Boehme and Ciaranello, 1982; Cabib et al., 1998; Erwin et al., 1993; Kanes et al., 1993; Ng et al., 1994), and D2R-like binding is lower (Ng et al., 1994; Cabib et al., 1998) or similar (receptor binding and mRNA) (Kanes et al., 1993) (Ng et al., 1994) in substantia nigra/ventral tegmentum area (SN/VTA) of C57BL/6 relative to DBA/2 mice. Similar D2R-like binding levels have been observed in the frontal cortex of C57BL/6 and DBA/2 mice (Erwin et al., 1993; Ng et al., 1994). Together, these findings suggest that strain differences in mesolimbic dopamine neurotransmission may be mediated in part by differences in D2R-family expression. However, the D2R ligands used in previous studies do not differentiate D2R and D3R binding, and no studies to date have specifically examined D3R binding/expression in these two mouse strains.

Evidence from pharmacological, antisense knockdown, and D3R mutant mouse studies suggest that the D3R plays an inhibitory role in the regulation of rodent locomotor activity, in opposition to the behavioral and intracellular signaling effects of concurrent D1 and D2 receptor stimulation (Carr et al., 2002; De Boer et al., 1997; Ekman et al., 1998; Holmes et al., 2004; Kling-Petersen et al., 1995; Ouagazzal and Creese, 2000; Pritchard et al., 2003; Waters et al., 1993; Xu et al., 1997; Zhang et al., 2004). Based on these observations, we hypothesized that differences in D3R expression/function contribute to the differing behavioral responses to novelty, AMPH, and D1R stimulation in C57BL/6J and DBA/2J mice. We therefore determined brain regional D3R binding and D3R mRNA expression, and D1R binding in C57BL/6J and DBA/2J mice. We also determined the expression of D3nf mRNA, an alternatively spliced variant of the D3R which may modulate D3R function through inhibition of full-length D3 receptor dopamine binding (Elmhurst et al., 2000) and/or modulation of intra-

cellular localization (Karpa et al., 2000; *reviewed in* Richtand, 2006; Richtand et al., 2005). Strain differences in D3R-mediated inhibitory function were determined by measuring the effects of a selective D3R agonist and antagonist on novelty- and AMPH-stimulated locomotor activity. Furthermore, based on evidence of D3R-mediated opposition to D1R-stimulated gene expression in co-expressing neurons in the dorsal and ventral striatum (Schwartz et al., 1998; Zhang et al., 2004), and D1R-stimulated locomotor activity (Mori et al., 1997; Starr and Starr, 1995; Xu et al., 1997), the locomotor response to the D1R-family partial agonist $\pm$ -1-phenyl-2,3,4,5-tetrahydro(1H)-3-benzazepine-7,8,-diol hydrochloride (SKF38393) was determined in both the presence and absence of D3R antagonist N-(4-[4-{2,3-dichlorophenyl}-1 piperazinyl]butyl)-2-fluorenylcarboxamide (NGB 2904) in C57BL/6J and DBA/2J mice. We report that C57BL/6J mice exhibit lower D3R binding in the ventral striatum, lower D3R mRNA expression in SN/VTA, and lower D3R-mediated inhibitory functional opposition to D1R-mediated locomotor activity relative to DBA/2J mice. These data demonstrate that C57BL/6J mice exhibit lower D3R-mediated inhibitory function relative to DBA/2J mice, and suggest that lower D3R-mediated inhibitory function may contribute to the more active phenotype of the C57BL/6J mouse strain.

EXPERIMENTAL PROCEDURES

Animals

Adult (8–9 weeks old) male C57BL/6J and DBA/2J mice were purchased from Jackson Laboratories (Bar Harbor, MA, USA), and allowed to acclimate to their home cage for at least 1 week prior to behavioral testing/tissue collection. Mice were housed in groups of four to five per cage, with food and water available *ad libitum*. Mice were maintained under standard vivarium conditions on a 12-h light/dark cycle, and all behavioral testing and tissue collection were conducted during the light portion of the cycle. Naïve mice were used for each experiment. All experimental procedures were approved by the Cincinnati Department of Veteran Affairs, Institutional Animal Care and Use Committee and are in accordance with the guidelines of the National Institutes of Health. Every effort was made to minimize the number of animals used and their suffering.

D3R and D3nf mRNA expression

Naïve adult (8–9 weeks) male C57BL/6J ($n=10-20$) and DBA/2J ($n=10-20$) mice were killed by decapitation, brains extracted, and ventral striatum (NAc, islands of Calleja, and olfactory tubercle), hippocampus, prefrontal cortex, and midbrain (SN/VTA) isolated and flash frozen in liquid nitrogen. Frozen tissue from individual animals was homogenized (Caframo Model RZR1 homogenizer, Warton, ON, Canada) in 1.0 ml of Tri Reagent per 50–100 mg of tissue, and total RNA isolated and precipitated according to the manufacturer's instructions (Molecular Research Center, Cincinnati, OH, USA). Pellets were resuspended in nuclease-free water and total RNA concentrations determined by A_{260} measurements. Samples were then diluted in nuclease-free water to achieve a final concentration of 0.1 $\mu\text{g}/\mu\text{l}$. A second dilution of 0.01 $\mu\text{g}/\mu\text{l}$ was prepared for quantitation of the internal standard glyceraldehyde-3-phosphate dehydrogenase (GAPDH). D3R, D3nf, and GAPDH mRNA expression were determined by real-time reverse transcriptase polymerase chain reaction (RT-PCR) as described by others (Medhurst et al., 2000). Primers and fluorogenic probes were designed using Primer Express v.2.0 software (Applied Bio-systems, Foster City, CA, USA) based on the mouse mRNA sequences for GAPDH (GenBank accession number M32599), D3R (accession number NM_007877), and D3nf determined from the full-length mouse D3R sequence using sequence homology to rat and human splice junctions. Primers used for GAPDH amplification were 5'-TGTTCCGTCGTGGATCTGA-3' and 5'-CCTGCTTCACCACCTTCTTGA-3' and the probe sequence was 5'-CCGCTGGAGAAACCTGCCAAGTATG-3'. The sequences of D3 primers were 5'-GAACTCCTTAAGCCCCACCAT-3' and 5'-

GAAGGCCCCGAGCACAAT-3' and the probe sequence was 5'-ACCCAAGCTCAGCTTAGAGGTTCTGA-3'. The D3nf primers were 5'-ACTCGGAAGCTCCTTAAGTACCACTTC-3' and 5'-GAAGGCCCCGAGCACAAT-3' and the probe was 5'-AGAAGAAGGCCACCCAGATGGTGG-3' (Midland Certified Reagent Company, Midland, TX, USA). Each probe was conjugated to a FAM reporter at the 5' end and a TAMRA quencher at the 3' end. A portion of the forward primer and the entire probe for D3R were located within the 98 nucleotide sequence which was deleted in D3nf, such that only the full length D3R cDNA was amplified by the D3R primers. The forward primer for D3nf spanned the splice junction and amplified only the D3nf cDNA. In this way, the primer sets uniquely amplified full length D3R or D3nf without overlap. The reverse primer for D3R and D3nf and the probe for GAPDH spanned exon-exon junctions to minimize genomic DNA contamination. RT-PCR was performed using a two-step methodology. A standard curve was constructed for each brain region by combining the total RNA of untreated C57BL/6J and DBA/2J mice. Standard curves consisted of seven points, using twofold, serial dilutions of known concentrations of total RNA. Standard curves were prepared from the same RNA samples for GAPDH, but at a 10-fold lower concentration. All reagents for real-time RT-PCR were obtained from Invitrogen, Carlsbad, CA, USA, unless otherwise stated. Reverse transcription was performed using the 9600 GeneAmp thermocycler (Perkin-Elmer, Norwalk, CT, USA). For each 50 μ l reaction, 10 μ l of diluted total RNA was mixed with 6.25 μ l random hexamer primers (100 μ M), 5 μ l PCR buffer (10X), 6 μ l MgCl₂ (25 mM), 2.5 μ l dNTP mix (10 mM), 0.5 μ l DTT (0.1M), 1.25 μ l RNaseOUT, 1.25 μ l MMLV RT and 17.25 μ l nuclease-free water (Ambion, Austin, TX, USA). For reverse transcription, samples were heated to 37 °C for 1 h and terminated by heating to 96 °C for 5 min. Each cDNA sample (10 μ l) was aliquoted into 96-well MicroAmp optical plates (Applied Biosystems, Foster City, CA, USA) in triplicate. A master mix of the following components was made and 40 μ l added to each sample for a total volume of 50 μ l (4 μ l PCR Buffer, 1 μ l dNTP mix, 2.8 μ l MgCl₂ for D3R and GAPDH, 6.8 μ l MgCl₂ for D3nf), 1 μ l of forward and reverse primers (50 μ M), 1.25 μ l probe (10 μ M), 1 μ l ROX (50 $\times$) (BioRad, Hercules, CA, USA), 0.5 μ l Platinum *Taq* polymerase and 27.45 μ l of water for D3R and GAPDH and 23.45 μ l for D3nf. Cycling parameters were as follows in a 7500 Real-Time PCR Detection System (Applied Biosystems): 95 °C for 10 min (hot start), 95 °C for 30 s (melting), 60 °C for one minute (annealing and extension). The last two steps were repeated for 45 cycles. A plot of threshold cycle (C_t, defined as the cycle during which probe fluorescence reached 10 times baseline) vs. log input RNA quantity was prepared for each standard curve. Linear regression analysis was performed using the 7500 Sequence Detection Software version 1.2.3 (Applied Biosystems). Relative quantities for D3R, D3nf and GAPDH mRNA in unknown samples were interpolated from the regression line for the corresponding standard curve, based on the median C_t value for each unknown sample. The relative quantities for D3R and D3nf were normalized to the GAPDH value obtained from the same mouse.

Dopamine D1 and D3 receptor binding

Naïve adult (8–9 weeks) male C57BL/6J and DBA/2J mice were killed by decapitation, brains extracted and frozen on dry ice. Ventral striata (NAc, islands of Calleja, and olfactory tubercle) were isolated by freehand dissection on ice. In order to provide sufficient material for accurate determination of both K_D and B_{max} values, tissues were pooled to yield six independent samples from each mouse strain for each receptor studied.

D1R-family (D1R, D5R) binding was quantified using [³H]SCH 23390 as previously described (Levant, 2003). Ventral striatal membranes were prepared in D1R assay buffer (50 mM Tris-HCl, 5 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, and 120 mM NaCl, pH 7.4 at 23 °C) to yield a final concentration of 20 μ g protein/tube. [³H]SCH 23390 (87 Ci/mmol; Amersham, Arlington Heights, IL, USA) was used at six concentrations (0.05–1.5 nM). Nonspecific binding was

defined in the presence of 1 μM (+)-butaclamol (Sigma Chemical Co., St. Louis, MO, USA). Binding assays were performed in duplicate in polystyrene tubes in a final volume of 0.5 ml. Binding was initiated with the addition of membrane homogenate and incubated for 90 min at 23 °C. The reaction was terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester using ice-cold wash buffer (50 mM Tris-HCl; pH 7.4 at 23 °C). Bound radioactivity was quantified using a Beckman scintillation counter.

D3R binding was quantified in separate samples using [^3H]PD 128907 as previously described (Bancroft et al., 1998). Under the *in vitro* assay conditions employed, [^3H]PD 128907 exhibits negligible binding at striatal D2R sites and thus has greater than 300-fold D3/D2 selectivity (Bancroft et al., 1998). The assay was performed as described for [^3H]SCH 23390 binding except the membrane was prepared in D3R assay buffer (50 mM Tris, 1 mM EDTA; pH 7.4 at 23 °C) to yield a final concentration of 200 μg protein/tube. Greater protein was required to assay D3R versus D1R because of the more than 10-fold difference in the level of expression of these receptors in the ventral striatum. Six concentrations of (+)-[N-propyl-2,3- ^3H]PD 128907 (114 Ci/mmol; Amer-sham) were used (0.04–1.4 nM). Nonspecific binding was defined by 1 μM spiperone (Sigma Chemical Co.). Assay tubes were incubated for 3 h at 23 °C.

Protein concentrations were determined using the BCA colorimetric assay method (Pierce, Rockford, IL, USA). Specific [^3H]SCH 23390 and [^3H]PD 128907 binding were expressed as fmol/mg protein, and D1R and D3R binding data were analyzed using SigmaPlot 8.0.

Drugs

(+)-PD 128907 hydrochloride (Tocris, Ballwin, MO, USA) was dissolved in sterile 0.9% NaCl. The 10 $\mu\text{g}/\text{kg}$ dose of PD128907 was selected because it was previously shown to selectively reduce novelty-induced locomotor activity in wild-type mice but not D3R knockout mice (Pritchard et al., 2003). NGB 2904 (Yuan et al., 1998) was dissolved in 50:50 polyethylene glycol (400):0.9% NaCl. NGB 2904 has 830-fold selectivity for rat D3R vs. D2R expressed in Sf9 cells (Newman et al., 2003), 155-fold selectivity for cloned primate D3R vs. D2R (Yuan et al., 1998), and 60-90-fold selectivity for human D3R vs. D2R (Grundt et al., 2005). NGB 2904 has greater than 3500-fold selectivity for D3R vs. D4R, 160-fold selectivity for D3R vs. rat 5-HT₂ receptor, and negligible affinity at other binding sites, including α_1 adrenergic receptors, in a global screen for other receptor binding sites (Yuan et al., 1998). D-AMPH sulfate (Research Biochemicals, Int., Natick, MA, USA) was dissolved in sterile 0.9% NaCl, and drug concentration is described as free base. SKF38393 (NIMH Chemical Synthesis and Drug Supply Program) was dissolved in sterile 0.9% NaCl. SKF38393 was chosen specifically because it was previously demonstrated to stimulate *c-fos* expression in ventral striatum neurons in a manner that was opposed by D3R-selective agonists (Schwartz et al., 1998; Ridray et al., 1998). Control injections consisted of an equivalent volume of the drug vehicle, and all injections were administered s.c. in a volume of 1.0 ml/kg.

Locomotor activity

Behavioral testing was performed in 28 automated residential activity chambers (RACS), each consisting of a lighted, ventilated, sound-attenuated cabinet housing a 40×40×38 cm Plexiglas enclosure. An air evacuation fan in each enclosure provided constant air circulation and background noise. Horizontal locomotor activity was monitored with a 16×16 photo beam array (San Diego Instruments, San Diego, CA, USA), and expressed as distance traveled per unit time interval. Mice were transported from the adjacent vivarium to the activity chamber room 30 min prior to placement into activity chambers. All behavioral testing was initiated between 10:00 a.m. and 12:00 p.m. Drug (PD128907 and NGB 2904) effects on novelty-induced locomotor activity were examined in mice naïve to the activity chambers, and drug

was administered immediately prior to placement into novel activity chambers. Drug (AMPH and SKF38393) effects on acclimated locomotor activity were examined in mice that had been habituated to the chambers for 3 h prior to drug administration. In experiments examining the effects of NGB 2904 on AMPH- and SKF38393-induced locomotor activity, NGB 2904 was administered 30 min prior to AMPH and SKF38393 treatment. The duration of post-treatment locomotor activity measured varied in accordance with the duration of specific drug effects on locomotor activity.

Statistical analysis

Statistical analyses were performed using GB-STAT (Dynamic Microsystems, Inc., Silver Springs, MD, USA). Behavioral (Distance) data were analyzed with a three-way ANOVA, with Strain (C57BL/6J, DBA/2J) and Drug dose (saline, drug doses) as main factors, and Time interval as the repeated measure, and individual group differences were assessed with Fisher's LSD tests ($\alpha=0.05$). Strain differences in D3R and D1R binding ($B_{\max}$ and K_D) and D3R/GAPDH and D3nf/GAPDH mRNA expression were assessed by Student's *t*-test (two-tailed, $\alpha=0.05$).

RESULTS

D3R and D3nf mRNA expression

D3R and D3nf mRNA expression were determined in prefrontal cortex, hippocampus, ventral striatum (NAc and islands of Calleja), and midbrain (SN/VTA) of drug-naïve C57BL/6J ($n=10-20$) and DBA/2J ($n=10-20$) mice, as illustrated in Fig. 1. D3R and D3nf mRNA expression were greater in the hippocampus (D3R, $+40\% \pm 3.5\%$, $P \leq 0.0001$; D3nf $+39\% \pm 5.9\%$, $P=0.0002$) of C57BL/6J relative to DBA/2J mice. D3R mRNA expression was lower in the SN/VTA ($-26\% \pm 2.8\%$ ($P=0.002$)) of C57BL/6J relative to DBA/2J mice. There were no strain differences in D3R or D3nf mRNA expression in ventral striatum (D3R $P=0.682$; D3nf $P=0.071$) or prefrontal cortex (D3R, $P=0.578$; D3nf, $P=0.719$), and in D3nf expression in the SN/VTA ($P=0.244$). The D3R:D3nf mRNA ratio did not differ between strains in the ventral striatum ($P=0.156$), SN/VTA ($P=0.284$), hippocampus ($P=0.859$), or prefrontal cortex ($P=0.121$), though there was a trend toward higher (+31%) D3:D3nf mRNA ratio in the PFC of DBA/2J relative to C57BL/6J mice (Fig. 1C).

D1R and D3R binding

Dopamine D1R and D3R binding ($B_{\max}$ and K_D) were determined in the ventral striatum (NAc and islands of Calleja) of drug-naïve C57BL/6J and DBA/2J mice (Fig. 2). Binding density ($B_{\max}$) for the D3R agonist [3 H]PD128907 was lower by 32% ($P \leq 0.01$), while the K_D was slightly lower ($P \leq 0.05$) in C57BL/6J compared with DBA/2J mice (Fig. 2A, legend). In contrast, binding density ($B_{\max}$, $P=0.453$) and affinity (K_D , $P=0.370$) for the D1R-family agonist [3 H]SCH23390 did not differ significantly between the two strains (Fig. 2B, legend).

Locomotor response to novelty

C57BL/6J mice exhibited significantly greater locomotor activity relative to DBA/2J mice beginning ~30 min following initial placement into novel activity chambers (see Fig. 3). Significant main effects of Strain, $F(1,17)=5.30$, $P=0.035$, and Time, $F(19,342)=39.6$, $P \leq 0.0001$, were found, and the Strain $\times$ Time interaction was significant, $F(19,323)=2.23$, $P=0.0026$.

Effect of (+)-PD128907 on novelty-induced locomotion

The effect of the D3R agonist (+)-PD128907 (10 μ g/kg) on novelty-induced locomotion was determined in C57BL/6J and DBA/2J mice (Fig. 4). Significant main effects of Strain, $F(1,55)$

$=3.87$, $P=0.05$, Treatment, $F(1,55)=8.96$, $P=0.004$, and Time Interval $F(4,208)=44.8$, $P\leq 0.0001$, were identified, and the Strain $\times$ Time Interval interaction, $F(4,208)=10.5$, $P\leq 0.0001$, was significant. The Strain $\times$ Treatment interaction, $F(1,55)=0.68$, $P=0.42$, and Treatment $\times$ Time Interval interaction, $F(4,208)=2.26$, $P=0.06$, were not significant. Locomotion was significantly reduced in the DBA/2J (+)-PD128907 group relative to the DBA/2J vehicle group ($P\leq 0.01$), the C57BL/6J vehicle group ($P\leq 0.01$), and the C57BL/6J (+)-PD128907 group ($P\leq 0.01$) (Fig. 4A, B, C). The similar level of novelty-induced locomotor activity displayed by saline-treated C57BL/6J and DBA/2J mice over the first 30 min was also observed in a separate group of naïve mice (Fig. 3). Moreover, the magnitude of the decrease in locomotor activity in DBA/2J mice ($\sim 27\%$) in response to treatment with (+)-PD128907 (10 $\mu\text{g/kg}$) is similar to that previously observed in wild-type mice ($\sim 20\%$) (Pritchard et al., 2003).

Effect of NGB 2904 on novelty-induced locomotion

The effect of the D3R antagonist NGB 2904 (0.01, 0.1, and 1.0 mg/kg) pretreatment (30 min prior in home cage) on novelty-induced locomotion in C57BL/6J and DBA/2J mice is shown in Fig. 5. A significant main effect of Time Interval, $F(4,188)=79.8$, $P\leq 0.0001$, was found, and the Strain $\times$ Time Interval interaction, $F(4,188)=3.32$, $P=0.011$, was significant. However, main effects of Strain, $F(1,54)=3.14$, $P=0.08$, and Treatment, $F(3,54)=0.75$, $P=0.526$, were not significant, nor were the Strain $\times$ Treatment interaction, $F(3,54)=0.26$, $P=0.86$, and Treatment $\times$ Time Interval interaction, $F(12,188)=0.88$, $P=0.57$. Locomotion was significantly elevated in the DBA/2J NGB 2904 (1.0 mg/kg) group compared with the DBA/2J vehicle group ($P\leq 0.05$) (Fig. 5A, C), while locomotion was significantly decreased in the C57BL/6J NGB 2904 (0.1 mg/kg) group compared with the C57BL/6J vehicle group ($P\leq 0.05$) (Fig. 5B, C).

Effect of NGB 2904 pretreatment on acute AMPH-induced locomotor activity

The effect of NGB 2904 pretreatment (0, 0.01, 0.1, or 1.0 mg/kg 30 min prior) on AMPH-stimulated locomotor activity (1.0 mg/kg) was determined in C57BL/6J and DBA/2J mice (Fig. 6). A significant main effect of Strain, $F(1,54)=20.1$, $P\leq 0.0001$, and Time Interval, $F(9,423)=27.3$, $P\leq 0.0001$ was found, whereas the main effect of NGB 2904 Treatment was not significant, $F(3,54)=0.05$, $P=0.98$. Neither the Strain $\times$ Treatment interaction, $F(3,54)=0.51$, $P=0.68$, nor the Treatment $\times$ Time Interval interaction, $F(27,423)=0.583$, $P=0.95$, was significant. Post hoc tests found that locomotor activity in the DBA/2J NGB 2904 (0.1 mg/kg) group was significantly greater than the DBA/2J vehicle group ($P\leq 0.05$) (Fig. 6A, C). In contrast, the C57BL/6J vehicle group did not differ significantly from any of the C57BL/6J NGB 2904 dose groups (Fig. 6B, C). Importantly, locomotor activity was significantly greater in the C57BL/6J vehicle group compared with the DBA/2J vehicle group ($P\leq 0.01$), confirming elevated AMPH-stimulated loco-motor activity in C57BL/6J relative to DBA/2J mice.

SKF38393-induced locomotor activity

The effect of the D1R-family partial agonist SKF38393 (0, 5, 10, and 20 mg/kg) on locomotor activity in chamber-acclimated C57BL/6J and DBA/2J mice is shown in Fig. 7. Significant main effects of Strain, $F(1,6)=29.1$, $P=0.001$, Treatment, $F(3,18)=57.3$, $P\leq 0.0001$, and Time Interval, $F(25,150)=11.3$, $P\leq 0.0001$, were observed. The Strain $\times$ Treatment interaction, $F(3,18)=3.82$, $P=0.028$, and Treatment $\times$ Time Interval interaction, $F(75,450)=2.11$, $P\leq 0.0001$, were significant, whereas the Strain $\times$ Time Interval interaction, $F(25,150)=1.21$, $P=0.24$, was not. Post hoc tests demonstrated that locomotor activity was significantly greater in C57BL/6J mice treated with all doses of SKF38393 compared with vehicle-treated C57BL/6J mice ($P\leq 0.001$). Locomotor activity differed significantly between the DBA/2J 5 mg/kg dose and both the DBA/2J 10 mg/kg ($P\leq 0.001$) and DBA/2J 20 mg/kg ($P\leq 0.001$) dose groups (Fig. 7A, C). In contrast, for C57BL/6J mice post hoc tests indicated that while loco-motor activity was elevated at each SKF38393 dose compared with vehicle-treated controls ($P\leq 0.001$), there

were no significant locomotor activity differences between SKF38393 doses ($P_s > 0.05$) (Fig. 7B, C). Of note, locomotor activity was significantly greater in C57BL/6J relative to DBA/2J mice at both the SKF38393 5 and 10 mg/kg doses ($P_s \leq 0.05$).

Effect of NGB 2904 pretreatment on SKF38393-induced locomotor activity

The effect of pretreatment with the D3R antagonist NGB 2904 on SKF38393 (1.0 mg/kg) - induced locomotor activity in C57BL/6J and DBA/2J mice is shown in Fig. 8. Significant main effects of Strain, $F(1,55)=19.5$, $P \leq 0.0001$, and Time Interval, $F(9,432)=15.1$, $P \leq 0.0001$, were found, and the Strain $\times$ Time Interval interaction was significant, $F(9,423)=9.75$, $P \leq 0.0001$. The main effect of Treatment, $F(3,55)=0.38$, $P=0.77$, and the Strain $\times$ Treatment interaction, $F(3,55)=0.35$, $P=0.79$, and the Treatment $\times$ Time Interval interaction, $F(27,432)=0.61$, $P=0.94$, were not significant. Post hoc tests demonstrate that locomotion is significantly elevated in the DBA/2J NGB 2904 (1.0 mg/kg) group compared with both the DBA/2J vehicle ($P \leq 0.05$) and DBA/2J NGB 2904 (0.01 mg/kg) groups ($P \leq 0.05$) (Fig. 8A, C). Locomotion was not significantly different in any of the C57BL/6J NGB 2904 dose groups compared with the C57BL/6J vehicle group ($P_s > 0.05$) (Fig. 8B, C).

DISCUSSION

In the present study, we determined D1R and D3R expression, and D1R and D3R behavioral function, in C57BL/6J and DBA/2J mice. We hypothesized that increased loco-motor responsiveness to novelty, D1R stimulation, and AMPH in C57BL/6 mice is mediated in part by deficits in D3R expression/function relative to DBA/2 mice. The major findings of the present study are that C57BL/6J and DBA/2J mice differ significantly in both D1R and D3R modulation of behavior. Deficits in D3R function in C57BL/6J mice relative to DBA/2J mice were evidenced by a diminished sensitivity to the locomotor-depressing effects of the D3R agonist (+)-PD128907, and diminished sensitivity to the locomotor-augmenting effects of the D3R antagonist NGB 2904. Indeed, NGB 2904 reduced novelty-induced locomotor activity in C57BL/6J mice, possibly as a result of NGB 2904 antagonist effects on D2R. Because D2R are more abundantly expressed than D3R, even limited effects on D2R could be apparent with diminished D3R function. We also demonstrate that C57BL/6J mice exhibit greater SKF38393-induced locomotor activity relative to DBA/2J mice, and that SKF38393-induced locomotor activity was augmented by the D3R antagonist NGB 2904 in DBA/2J mice but not in C57BL/6J mice. When coupled with prior evidence of D3R-mediated opposition to D1R-stimulated locomotor activity (Mori et al., 1997; Starr and Starr, 1995; Xu et al., 1997), these data also suggest that D3R function is lower in C57BL/6J mice relative to DBA/2J mice.

The present study also confirmed and extended the findings of others that acute AMPH produced greater locomotor activity in C57BL/6J mice than DBA/2J mice (Cabib, 1993; Puglisi-Allegra and Cabib, 1997; Zocchi et al., 1998), and demonstrated that the D3R antagonist NGB 2904 augmented acute AMPH-induced locomotor activity in DBA/2J mice, but not in C57BL/6J mice. These findings lend further support for the idea that diminished D3R-mediated inhibitory opposition to D1R signaling in C57BL/6J mice may play an important role in this strain's heightened sensitivity to dopamine-stimulating drugs. Moreover, these data extend previous findings to suggest that C57BL/6J mice exhibit differences in both D2R as well as D3R expression/function relative to DBA/2J mice (*reviewed in* Cabib et al., 2002; Puglisi-Allegra and Cabib, 1997).

Efforts to elucidate the specific mechanisms through which D3R-mediated processes contribute to behavioral differences between genotypes are described below. D3R expression differed between C57BL/6J and DBA/2J mice in ventral striatum, with reduced $B_{\max}$ in C57BL/6J mice and a lower K_D in DBA/2J mice. These differences would therefore translate into lower D3R binding in C57BL/6J mice under conditions of saturating dopamine

concentrations, but could result in increased D3R function in C57BL/6J mice at non-saturating dopamine concentrations. In contrast, D1R binding did not differ between strains. The absence of a strain difference in D1R binding in the ventral striatum is consistent with previous reports (Erwin et al., 1993; Cabib et al., 1998), and would result in an elevated D1R:D3R ratio under saturating dopamine concentrations within the ventral striatum in C57BL/6J relative to DBA/2J mice. D3R are co-expressed with D1R in the majority of neurons within the ventral striatum (Diaz et al., 2000; Le Moine and Bloch, 1996; Ridray et al., 1998; Surmeier et al., 1996), and D3R and D1R interact in an antagonistic manner in the regulation of *c-fos* expression in dorsal and ventral striatum neurons (Schwartz et al., 1998; Ridray et al., 1998; Zhang et al., 2004). C57BL/6J mice exhibit greater AMPH- and D1R agonist-induced Fos protein expression in the NAc relative to DBA/2J mice (Conversi et al., 2004). Furthermore, D3R and D1R interact in an antagonistic manner in the regulation of locomotor activity (Mori et al., 1997; Starr and Starr, 1995; Xu et al., 1997). Collectively, these findings are consistent with the hypothesis that lower D3R-mediated opposition to D1R-signaling in the ventral striatum contributes to the greater locomotor responsiveness to novelty-, D1R agonist- and AMPH-stimulated locomotion in C57BL/6J relative to DBA/2J mice.

D3R mRNA expression was lower (−26%) in the SN/VTA region of C57BL/6J relative to DBA/2J mice, while D3R mRNA expression in ventral striatum (NAc, islands of Calleja) and prefrontal cortex was similar in the two mouse strains. In combination, these findings suggest the possibility of reduced D3 autoreceptor expression at somatodendritic and/or axon terminal regions in the mesocorticolimbic and nigrostriatal neuronal pathways. D3 autoreceptor function, while somewhat controversial (Joseph et al., 2002; Koeltzow et al., 1998; Zapata et al., 2001), has been previously demonstrated through antisense knockdown of D3R expression in rat SN neurons, which decreased inhibitory feedback produced by apomorphine, but did not alter spontaneous neuronal activity (Tepper et al., 1997). In rat brain, D3R immunoreactivity is found in the majority of tyrosine hydroxylase-positive (dopaminergic) neurons in the SN/VTA (Diaz et al., 2000; Khan et al., 1998), and a growing body of evidence supports the existence of somatodendritic and axonal D3 autoreceptors on SN/VTA neurons (Diaz et al., 2000; Joseph et al., 2002; Kling-Petersen et al., 1995; Tepper et al., 1997; Zapata et al., 2001). Differences in autoreceptor function between C57BL/6J and DBA/2J mice are suggested by the observations that AMPH-induced increases in extracellular dopamine levels in the NAc are significantly greater in C57BL/6 than in DBA/2J mice, whereas basal extracellular dopamine levels do not differ between strains (Ventura et al., 2004a,b; Zocchi et al., 1998). In combination, these findings suggest that C57BL/6J mice exhibit diminished D3 autoreceptor function in SN/VTA neurons relative to DBA/2J mice. More detailed studies will be required to elucidate the degree to which D3 autoreceptor and/or post-synaptic mechanisms contribute to the behavioral differences between C57BL/6J and DBA/2J mice.

C57BL/6J mice exhibited greater (+40%±3.5%) D3R mRNA expression in the hippocampus relative to DBA/2J mice. The hippocampus, particularly the CA1 region, receives afferent dopaminergic projections from a subpopulation of SN/VTA neurons (Gasbarri et al., 1994) and, within the rat hippocampus, D3R mRNA is expressed by dentate gyrus granule cells and CA1 pyramidal neurons (Bouthenet et al., 1991), and D3R immunoreactivity is restricted to the inner third of the dentate gyrus molecular layer and stratum oriens and radiatum fields of CA1 (Khan et al., 1998). A previous study found that hippocampal D1R binding is similar between strains (Ng et al., 1994), suggesting that C57BL/6J mice exhibit a lower D1R:D3R ratio in the hippocampus relative to DBA/2J mice. Although the functional role of D3R in the hippocampus is unknown, D1R activation is required for the maintenance phase of CA1 long-term potentiation (Huang and Kandel, 1995) and the D3R may exert inhibitory opposition to D1R-mediated signaling in the regulation of hippocampal synaptic plasticity (Hammad and Wagner, 2006). However, C57BL/6J mice exhibit persistent hippocampal long-term potentiation, whereas the maintenance phase of hippocampal long-term potentiation is

impaired in DBA/2 mice (Matsuyama et al., 1997). Furthermore, C57BL/6J mice exhibit superior performance on hippocampal-dependent learning tasks relative to DBA/2J mice (e.g. Paylor et al., 1994). Therefore, while the precise functional role of the D3R in the hippocampus remains to be elucidated, it is possible that decreased D3R expression in the hippocampus of DBA/2J mice contributes to deficits in hippocampal synaptic plasticity and function in this mouse strain.

Acute AMPH treatment stimulates greater extracellular dopamine levels in medial PFC of DBA/2J mice relative to C57BL/6J mice (Ventura et al., 2004b). In the present study, we did not observe a significant difference in D3 mRNA expression in the PFC, though we did observe a trend toward a higher D3:D3nf mRNA ratio (+31%) in the PFC of DBA/2J relative to C57BL/6J mice. In view of the potential role of D3nf in the modulation of D3R function and/or intracellular localization (Elmhurst et al., 2000; Karpia et al., 2000; Richtand et al., 2005; Richtand, 2006), these findings may indicate potential differences in medial PFC D3R intracellular localization in C57BL/6J and DBA/2J mice. Future studies examining D3R expression/function in medial PFC of C57BL/6J and DBA/2J mice are therefore warranted.

D3nf is an alternatively spliced, truncated, cytoplasmic D3R isoform which dimerizes with the full-length D3R, reducing D3R ligand binding and promoting full-length D3R internalization (Elmhurst et al., 2000; Karpia et al., 2000; Liu et al., 1994). Of interest, D3nf mRNA splicing efficiency is elevated, and D3R mRNA expression reduced, in postmortem cortex of schizophrenia patients (Schmauss, 1996). D3nf mRNA expression is also elevated in the hippocampus of C57BL/6J mice relative to DBA/2J mice, but did not differ significantly between strains in the prefrontal cortex, ventral striatum, or SN/VTA regions. Because hippocampal D3R and D3nf mRNA levels were both elevated in C57BL/6J mice relative to DBA/2J mice, the D3:D3nf mRNA ratio did not differ significantly between strains in any brain region examined. Further study is needed to clearly elucidate the functional role of D3nf.

Collectively, these data suggest that lower D3R expression/function may contribute to heightened sensitivity to the locomotor-stimulating, and potentially the sensitizing and reinforcing, effects of AMPH, and suggest that contrasting C57BL/6J and DBA/2J mouse strains may represent an important paradigm to further elucidate the genetic and environmental factors that contribute to phenotypic differences in mesocorticolimbic dopamine neurotransmission.

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Abbreviations

AMPH, amphetamine; Ct, threshold cycle; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NAc, nucleus accumbens; NGB 2904, N-(4-[4-{2,3-dichlorophenyl}-1 piperazinyl]butyl)-2-fluorenylcarboxamide; RT-PCR, reverse-transcriptase polymerase chain reaction; SKF38393, $\pm$ -1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8,-diol hydrochloride; SN, substantia nigra; VTA, ventral tegmentum area.

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Fig. 1.

D3R mRNA expression (A), D3nf mRNA expression (B), and D3R:D3nf mRNA ratios (C) in the prefrontal cortex (PFC), ventral striatum (Nac/islands of Calleja, IC), midbrain (SN/VTA), and hippocampus (HPC) of C57BL/6J ($n=10-20$) and DBA/2J ($n=10-20$) mice. All D3R and D3nf mRNA values are normalized to GAPDH mRNA. Note: (1) D3R mRNA expression is significantly greater in the SN/VTA of DBA/2J mice relative to C57BL/6J mice, and (2) D3R and D3nf mRNA expression are significantly lower in the hippocampus of C57BL/6J mice relative to DBA/2J mice. There were no significant strain differences in D3R:D3nf mRNA ratio in any brain region. Data are expressed as group mean $\pm$ S.E.M. ** $P\leq 0.01$ vs. C57BL/6J mice, *** $P\leq 0.001$ vs. DBA/2J mice.

Fig. 2.

Rosenthal plots of (A) D3R binding ([³H]PD128907) and (B) D1R binding ([³H]SCH23390) in the ventral striatum of DBA/2J and C57BL/6J mice. Membranes were incubated with six concentrations of [³H]PD128907 (0.04–1.4 nM) or [³H]SCH23390 (0.05–1.5 nM) ($n=6$ independent pooled samples for each assay). For D3R binding, the $B_{\max}$ value for C57BL/6J mice (26 ± 1.7 fmol/mg protein) was significantly lower than in DBA/2J mice (38 ± 2.7 fmol/mg protein) ($P < 0.01$). The K_D value for C57BL/6J mice ($0.41 \text{ nM} \pm 0.04 \text{ nM}$) was significantly lower than for DBA/2J mice ($0.71 \text{ nM} \pm 0.11 \text{ nM}$) ($P < 0.05$). For D1R binding, C57BL/6J mice exhibited a $B_{\max}$ of 503 ± 25 fmol/mg protein and a K_D value of $0.14 \text{ nM} \pm 0.021 \text{ nM}$ and DBA/2J mice exhibited a $B_{\max}$ of 506 ± 45 fmol/mg protein and a K_D value of $0.18 \text{ nM} \pm 0.033 \text{ nM}$. D1R $B_{\max}$ and K_D values did not differ significantly between strains (B/T, specific binding/total ligand concentration).

Fig. 3. Locomotor activity of naïve C57BL/6J ($n=9$) and DBA/2J ($n=9$) mice following placement in a novel activity chamber. Note that C57BL/6J mice exhibit significantly greater novelty-induced locomotor activity relative to DBA/2J mice. Data are expressed as group mean $\pm$ S.E.M. ** $P\leq 0.01$ vs. DBA/2J mice.

Fig. 4.

Effect of the D3R agonist (+)-PD128907 (PD, 10 µg/kg) or saline (1 ml/kg) pretreatment on novelty-induced locomotor activity in (A) DBA/2J ($n=14/\text{group}$) and (B) C57BL/6J ($n=14/\text{group}$) mice following placement into novel activity chambers. Mean total distance data collapsed over the first 30 min is presented in (C). Note that PD significantly reduces novelty-induced locomotor activity to greater extent in DBA/2J mice relative to C57BL/6J mice. Data are expressed as group mean $\pm$ S.E.M. * $P\leq 0.05$, ** $P\leq 0.01$ vs. saline-treated mice in (A, B), ** $P\leq 0.01$, PD-treated C57BL/6J mice vs. PD-treated DBA/2J mice (C) determined by repeated measures ANOVA and Fisher LSD post hoc comparisons.

Fig. 5.

Effect of pretreatment with the D3R antagonist NGB 2904 (0.01, 0.1, 1.0 mg/kg) or drug vehicle (1 ml/kg) on novelty-induced locomotor activity of naïve (A) DBA/2J ($n=7$ /group) and (B) C57BL/6J ($n=7$ /group) mice following placement into novel activity chambers. Mean total distance data collapsed over the first 45 min is presented in (C). Note that pretreatment with NGB 2904 (1.0 mg/kg) increased novelty-induced locomotor activity in DBA/2J mice, and (0.1 mg/kg) decreased novelty-induced locomotor activity in C57BL/6J mice. Data are expressed as group mean $\pm$ S.E.M. * $P \leq 0.05$ vs. same strain vehicle-treated control determined by repeated measures ANOVA and Fisher LSD post hoc comparisons.

Fig. 6.

Effect of pretreatment with the D3R antagonist NGB 2904 (0.01, 0.1, 1.0 mg/kg) or drug vehicle (1 ml/kg) on acute AMPH (1 mg/kg)-induced locomotor activity in (A) DBA/2J ($n=7$ /group) and (B) C57BL/6J ($n=7$ /group) mice. Mean total distance data collapsed over the first 90 min is presented in (C). Note that NGB 2904 (0.1 mg/kg) increases AMPH-induced locomotor activity in DBA/2J mice but had little effect in C57BL/6J mice. Data are expressed as group mean $\pm$ S.E.M. * $P\leq 0.05$ vs. vehicle-treated DBA/2J mice determined by repeated measures ANOVA and Fisher LSD post hoc comparisons. $^{\dagger}P\leq 0.001$ vs. vehicle-treated DBA/2J mice determined by repeated measures ANOVA and Fisher LSD post hoc comparisons. Note different y axis scales in (C).

Fig. 7.

Effect of the D1R agonist SKF38393 (5, 10, 20 mg/kg) or drug vehicle (1 ml/kg) on locomotor activity in (A) DBA/2J ($n=7$ /group) and (B) C57BL/6J ($n=7$ /group) mice. Mean total distance data collapsed over the first 180 min is presented in (C). Note that SKF38393 increases locomotor activity in both DBA/2J and C57BL/6J mice, and that SKF38393-induced locomotor activity is greater in C57BL/6J mice. Data are expressed as group mean $\pm$ S.E.M. *** $P\leq 0.001$ vs. same strain vehicle-treated mice determined by repeated measures ANOVA and Fisher LSD post hoc comparisons.

Fig. 8.

Effect of NGB 2904 pretreatment on SKF38393 (1 mg/kg) -induced locomotor activity in (A) DBA/2J ($n=7$ /group) and (B) C57BL/6J ($n=7$ /group) mice. NGB 2904 (0.01, 0.1, 1.0 mg/kg) or drug vehicle (1 ml/kg) pretreatment was given 30 min prior to SKF38393 in chamber-acclimated C57BL/6J and DBA/2J mice. Mean total distance data collapsed over the first 90 min is presented in (C). Note that NGB 2904 (1.0 mg/kg) increases SKF38393-induced locomotor activity in DBA/2J mice but not in C57BL/6J mice. Data are expressed as group mean $\pm$ S.E.M. * $P \leq 0.05$ vs. vehicle-treated DBA/2J mice determined by repeated measures ANOVA and Fisher LSD post hoc comparisons.