European Journal of Neuroscience, Vol. 26, pp. 3215–3228, 2007 doi:10.1111/j.1460-9568.2007.05888.x

Netrin-1 receptor-deficient mice show enhanced mesocortical dopamine transmission and blunted behavioural responses to amphetamine

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Keywords: axon guidance, DCC (deleted in colorectal cancer), prefrontal cortex, psychostimulants, UNC5H (UNC-5 homolgues)

Abstract

The mesocorticolimbic dopamine (DA) system is implicated in neurodevelopmental psychiatric disorders including schizophrenia but it is unknown how disruptions in brain development modify this system and increase predisposition to cognitive and behavioural abnormalities in adulthood. Netrins are guidance cues involved in the proper organization of neuronal connectivity during development. We have hypothesized that variations in the function of DCC (deleted in colorectal cancer), a netrin-1 receptor highly expressed by DA neurones, may result in altered development and organization of mesocorticolimbic DA circuitry, and influence DA function in the adult. To test this hypothesis, we assessed the effects of reduced DCC on several indicators of DA function. Using *in-vivo* microdialysis, we showed that adult mice that develop with reduced DCC display increased basal DA levels in the medial prefrontal cortex and exaggerated DA release in response to the indirect DA agonist amphetamine. In contrast, these mice exhibit normal levels of DA in the nucleus accumbens but significantly blunted amphetamine-induced DA release. Concomitantly, using conditioned place preference, locomotor activity and prepulse inhibition paradigms, we found that reduced DCC diminishes the rewarding and behavioural-activating effects of amphetamine and protects against amphetamine-induced deficits in sensorimotor gating. Furthermore, we found that adult DCC-deficient mice exhibit altered dendritic spine density in layer V medial prefrontal cortex pyramidal neurones but not in nucleus accumbens medium spiny neurones. These findings demonstrate that reduced DCC during development results in a behavioural phenotype opposite to that observed in developmental models of schizophrenia and identify DCC as a critical factor in the development of DA function.

Introduction

A combination of genetic and environmental events occurring during the prenatal or perinatal periods of life appears to lead to abnormalities in dopamine (DA) synaptic organization and neurotransmission in the medial prefrontal cortex (mPFC) as well as sensitized mesolimbic DA function later on in life. Such abnormalities have been linked to psychiatric disorders in humans, including schizophrenia, where under- or malfunction of mPFC DA contributes to cognitive symptoms and sensitized mesolimbic DA function contributes to psychotic symptoms. This suggests that early life events can result in subtle variations in the normal course of DA system development that, in turn, result in differential predisposition to cognitive and behavioural abnormalities in the adult (Weinberger, 1987; Lewis & Gonzalez-Burgos, 2000). How such early events might result in enduring changes in DA function is unknown. One possibility is through the reorganization of DA circuitry by altering the function of proteins involved in DA wiring. Netrin-1, a member of the mammalian netrin protein family, is a guidance cue that, by attracting or repelling growing axons, directs them toward their appropriate targets (Manitt & Kennedy, 2002). Here we report a study in which we investigated whether abnormal levels of the netrin-1 receptor DCC (deleted in colorectal cancer) during brain development can influence DA function and DA-related behaviours in the adult.

There are two reasons why we thought this influence might occur. First, there is evidence showing that DCC receptors are highly expressed in the mesocorticolimbic system and DA neurones in both the developing and adult brain (Gad *et al.*, 1997; Livesey & Hunt, 1997; Volenec *et al.*, 1998; Shu *et al.*, 2000; Lin *et al.*, 2005; Osborne *et al.*, 2005). DCC may therefore participate in the development, organization and maintenance of DA circuitry. Second, in a previous study we showed that adult *dcc* heterozygous mice (*dcc* homozygotes die at birth) exhibit sizeable increases in baseline tissue DA levels in the mPFC. Interestingly, these mice showed blunted amphetamine (AMPH)-induced locomotor activity, known to be mediated by drug-induced striatal DA release, and did not develop sensitization to this effect when treated repeatedly (Flores *et al.*, 2005). This may well

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Received 14 August 2007, revised 6 September 2007, accepted 13 September 2007

relate to the inhibitory effect that mesocortical DA activity exerts on mesolimbic DA activity (Grace, 1991; Le Moal & Simon, 1991). However, whether the increased tissue DA levels in the mPFC are associated with enhanced extracellular concentrations of DA within this region and are responsible for decreased mesolimbic DA activity remains to be examined.

Nevertheless, these studies suggest a link between netrin-1 and DA function and raise the interesting possibility that decreased function of the netrin-1 receptor DCC protects against the development of DA and behavioural abnormalities associated with schizophrenia-like symptoms. To determine whether this is the case, we tested for differences between adult *dcc* heterozygous and wild-type mice in a number of AMPH-induced behavioural effects that reflect the 'state' of functioning of the mesocorticolimbic system and that are known to be altered in schizophrenia and related disorders. To determine whether these behavioural changes are indeed accompanied by changes in DA responsiveness, we measured AMPH-induced DA release in the mPFC and nucleus accumbens (NAcc) by *in-vivo* microdialysis in freely moving animals.

Materials and methods

Animals

Adult male and female *dcc* heterozygous (+/–) mice, originally obtained from Dr S. Ackerman (The Jackson Laboratory) and maintained in the BL6 background at our animal colony, were used in all experiments. Mice were kept on a 12-h light/dark cycle with *ad-libitum* access to food and water. All behavioural testing was conducted during the light phase of the cycle. Pups were weaned at postnatal day 25 and housed in cages with same-sex littermates. Different cohorts of +/– and wild-type (+/+) mice were used for each experiment, counterbalancing for genotype, chamber assignment and treatment. All experiments were performed in accordance with the guidelines of the Canadian Council of Animal Care and all animal procedures were approved by the McGill University/Douglas Hospital Animal Care Committee and by the Animal Research Ethics Committee (AREC) of Concordia University.

Drugs

Amphetamine (AMPH; D -amphetamine sulphate salt (Sigma) was dissolved in 0.9% saline and was injected i.p.

Locomotor activity

Locomotor activity was quantified with an infrared activity monitoring apparatus modified for use with mice (AccuScan Instruments, Columbus, OH, USA) and was expressed as distance travelled (cm). Data collection occurred over 3 days. On day 1, mice were habituated to the boxes for 15 min; on day 2, mice were habituated to the boxes again for 15 min, then given a saline injection and returned to the boxes for an additional 30 min; on day 3, following the 15 min habituation period, mice were given an injection of AMPH (females, 2.2 mg/kg; males, 2.5 mg/kg; i.p.) and continued to be monitored for 90 min. These doses were based on our previous study where we conducted dose–response experiments on the locomotor effects of AMPH on adult male mice (Flores *et al.*, 2005) and were adjusted for females so as to produce equivalent drug brain concentrations (Becker *et al.*, 1982). Stereotypy counts were measured as the number of breakings of the same photocell beam or set of beams repeatedly, as defined by the AccuScan system.

Prepulse inhibition of acoustic startle response

Apparatus

Prepulse inhibition (PPI) was assessed using startle chambers (SR-LAB, San Diego Instruments, San Diego, USA) containing a clear Plexiglas cylinder that housed the animal during the testing session. Background white noise of 70 dB was delivered throughout the testing session to mask extraneous noise. The startle response for each trial was calculated as the mean of 65 readings taken at 1-ms intervals from stimulus onset. Prior to each testing session, the chambers were calibrated to ensure equivalent sensitivity to vibration and sound levels (C weighting).

Procedure

In all experiments, testing occurred over 4 days. On day 1, baseline measures of startle response and PPI were obtained. On days 2 and 3, male mice were left undisturbed in their home cages. On day 4, AMPH effects on PPI were tested. Each PPI session consisted of 12 startle, six prepulse and six no-stimulus (null) trials. Each session began with a 5-min acclimation of background noise followed by trials arranged in a pseudorandom order to prevent consecutive presentations of the same trial type. Startle trials consisted of presentation of a 40-ms/120-dB pulse. In the prepulse trials, the startle pulse was preceded (100 ms) by a 20-ms prepulse of varying intensity (5, 10 and 15 dB above background). The degree of PPI was calculated as a percentage for each prepulse intensity: $\%PPI = 100 \times [1 - (\text{mean prepulse trial} - \text{mean null})/(\text{mean startle} - \text{mean null})]$.

Two doses of AMPH were tested, 3.2 and 6.4 mg/kg ('low' and 'high', respectively). These doses were chosen on the basis of a previous study showing AMPH-induced PPI impairment in adult male BL6 mice (Tsai *et al.*, 2004). Please note that doses less than 3 mg/kg do not reliably induce deficits in PPI in BL6 mice (e.g. Varty *et al.*, 2001; Tsai *et al.*, 2004). The baseline measures of startle response and PPI obtained in the 'low' and 'high' experiment were combined.

Conditioned place preference

An automated three-compartment apparatus modified and adapted for use in mice was used with infrared photobeam detectors (ENV-013, MED Associates Inc., Vermont, USA) interfaced to a MED control system with MED-PC software. The two conditioning side compartments had distinctive visual and tactile cues that were balanced such that no side preference was exhibited before conditioning. Testing lasted for 5 days and consisted of three phases: preconditioning, conditioning and postconditioning test. On day 1 (preconditioning), female mice were allowed to move freely throughout all three compartments for 30 min and the time spent in each compartment was monitored. For the next three conditioning days, mice were exposed to twice-daily conditioning sessions. They were randomly assigned to receive AMPH pairings with one of the side compartments and saline pairings with the other compartment in a counterbalanced fashion. In the morning of each of the three conditioning days, they received saline and were confined to one compartment for 30 min. In the afternoon, they received AMPH (2.2 or 4.4 mg/kg, i.p.) in the other side compartment. These doses were based on our previous findings and on a previous report showing conditioned place preference (CPP) in adult male BL6 mice (Budygin *et al.*, 2004) and were adjusted for females. The postconditioning test was conducted on the fifth day when mice in a drug-free state were allowed to move freely between compartments for 30 min and the amount of time spent in each was recorded. This test was conducted between the time periods used previously for the morning and afternoon conditioning sessions.

In-vivo *microdialysis*

Surgery

Mice were anaesthetized using sodium pentobarbital (75 mg/kg, i.p.) and then given atropine sulphate (0.25 mg/kg, s.c.) to reduce bronchial secretions. Animals were mounted in a stereotaxic apparatus and a 21-gauge guide cannula was implanted into the NAcc (AP, +1.8 mm; ML, +0.8 mm; DV, −4.5 mm from the skull; Paxinos & Franklin, 2001) or into the mPFC (AP, +1.8 mm; ML, +0.6 mm; DV, −1.0 mm from the skull; Paxinos & Franklin, 2001). The cannula was held in place using Geristore adhesive (Den-Mat Corp., Santa Maria, CA, USA). Mice were allowed to recuperate in their home cages undisturbed for at least 5 days prior to microdialysis experiments.

Microdialysis

The tip of the microdialysis probes consisted of a semipermeable dialysis membrane (Spectra/Por, Spectrum Laboratories, Rancho Dominguez, CA, USA) with a molecular cut-off weight of 13 000 kDa. The total length of the tip of the microdialysis probes was 1 mm for the NAcc and 2 mm for the mPFC. Dialysate was collected from the probe outlet tubing (silica) into a 0.2-mL microcentrifuge tube. The night before the experiment, male mice were placed in a lidless cage to allow for habituation and the probes were connected to the pump. On testing day, probes were inserted into the intracranial cannula and then flushed with artificial cerebral spinal fluid (150 mM Cl^-, 145 mM Na^+, 2.7 mM K^+, 2 mM Na_2HPO_4, 1.22 mM Ca^{2+}, 1.0 mM Mg^{2+}, 0.2 mM ascorbate, pH 7.4 ± 0.1) at a rate of 0.05 mL/h (i.e. 0.83 μL/min) for 3 h prior to collection of samples. Four baseline 20-μL samples were collected every 20 min. Mice then received an injection of AMPH (2.5 mg/kg) and five additional samples were collected every 20 min. This dose was based on our locomotor studies in adult male mice (Flores *et al.*, 2005). Throughout the habituation period and during the testing procedure animals were freely moving and had *ad-libitum* access to food and water.

High-performance liquid chromatography

Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid were measured by electrochemical detection as previously described (Flores *et al.*, 2005). Data for DA, DOPAC and homovanilic acid were analysed through an EZChrom Chromatography Data System (Scientific Software, Inc., San Ramon, CA, USA).

Histology

Animals were given an overdose of sodium pentobarbital (>75 mg/kg, i.p.) and perfused intracardially with 0.9% saline and formaldehyde (10% formalin V/V, Anachemia, Montreal, QC, Canada). Brains were removed, frozen, and sectioned at 20 μm using a cryostat to determine the placement of dialysis probes. Probe placement was verified by assessment of Nissl-stained sections. Only data from animals with correct probe placement were used in the study.

Western blotting

The expression of DCC and UNC5H (UNC-5 homologues), the other netrin-1 receptor family, in the NAcc, mPFC and ventral tegmental area (VTA) of brains from male +/– and +/+ mice was measured through western blotting as described previously (Flores *et al.*, 2005). Briefly, animals were killed by decapitation following carbon dioxide anesthesia. Brains were then removed and rapidly frozen in 2-methylbutane (Fisher Scientific, Hampton, NH, USA) chilled with dry ice. Bilateral punches of mPFC, including cingulate cortex area 1 and 2, NAcc, including both core and shell, and VTA were excised from 1-mm-thick coronal sections. Sampling areas of mPFC and NAcc were taken starting from sections corresponding to Plate 15 and those of the VTA corresponding to Plate 55 of Paxinos & Franklin (2001). Protein samples (25 μg) were resolved using sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Amersham Biosciences, Freiburg, Germany). The membrane was incubated with antibodies against DCC (1 : 1000, mouse monoclonal, Cat. no. 554223, Pharmingen, Mississauga, Canada), UNC 5H (1 : 7500, rabbit polyclonal, kindly provided by Dr Tony Pawson, University of Toronto) and tubulin (1 : 4000, mouse monoclonal, Sigma). Bands were detected by chemiluminescence (Perkin Elmer, Waltham, MA, USA) and analysed using Kodak Imaging system software (2000, New Haven, CT, USA).

Immunofluorescence

Mice were anaesthetized with an overdose of sodium pentobarbital (> 75 mg/kg i.p.) and perfused intracardially with 50 mL of 0.9% saline followed by 80 mL of fixative solution (4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer). Mouse brains were dissected from the skull and postfixed in the same fixative for 45 min at 4 °C. Brains were then cryoprotected in sucrose (30% in phosphate-buffered saline) overnight at 4 °C. The following morning, tissue was rapidly frozen by immersion in 2-methylbutane chilled with dry ice. Frozen brains were immediately sectioned using a Leica SM2000-R sliding microtome.

Free-floating brain sections (40 μm) were processed for dual-labelling immunofluorescence. Briefly, sections were collected and rinsed in phosphate-buffered saline and incubated in blocking solution (2% bovine serum albumin, 0.2% Tween-20 in phosphate-buffered saline) for 1 h at room temperature (22°C). Sections were incubated overnight at 4 °C with combinations of primary antibodies diluted in blocking solution. The primary antibodies against DCC and UNC5H used in this procedure were the same as those described in the western blotting section. The two combinations of primary antibodies were as follows: (i) monoclonal anti-DCC (1 : 500) and polyclonal anti-tyrosine hydroxylase (TH) (1 : 500, raised in rabbit, Chemicon, Temecula, CA, USA, Cat. no. AB152), and (ii) polyclonal pan-UNC-5 (1 : 5000) and monoclonal anti-TH (1 : 300, Chemicon, Cat. no. MAB318). The sections were then washed several times in blocking solution and incubated with Alexa 488- and Alexa 546-conjugated secondary antibodies raised in goat (1 : 500, Molecular Probes, Eugene, OR, USA) for 45 min at room temperature. Immunofluorescence was visualized using a Leica DM4000B microscope and images were captured with a Microfire camera and PictureFrame software (Microbrightfield, VT, USA).

Golgi-Cox staining

Procedure

Adult male mice were given an overdose of sodium pentobarbital (> 75 mg/kg i.p.) and perfused transcardially with 0.9% saline. The brains were immersed in 20 mL of Golgi-Cox solution and stored (in the dark) in the Golgi-Cox fixative for 14 days before being transferred to a solution of 30% sucrose for 7 days. The tissue was cut into 200-μm-thick sections using a Vibratome™ and developed using a method described by Gibb & Kolb (1998).

Anatomical analysis

Because of the changes in DA function observed in +/– mice, dendritic spine density was analysed in neurones within the NAcc and mPFC that receive robust DA innervation, i.e. basilar dendrites of layer V of mPFC pyramidal neurones and NAcc medium spiny neurones (Fallon & Loughlin, 1987). In addition, to find out if changes in mPFC pyramidal neurones are specific to regions highly innervated by DA cells, we assessed dendritic spine density in layer III. Measurements were taken from the prelimbic subregion of the mPFC. We were also interested in analysing changes in layer III because, in postmortem brains of schizophrenic patients, there is a reduction in dendritic spine density in layer III pyramidal neurones of the dorsolateral prefrontal cortex (Glantz & Lewis, 2000).

A Leica model DM4000 microscope equipped with a Ludl XYZ motorized stage was used to identify cells, trace dendritic segments and quantify dendritic spines. Relevant regions were first identified at low magnification (250×). Only dendritic trees of a cell that was intact, well impregnated and not obscured by blood vessels, astrocytes or heavy clusters of dendrites from other cells were included in the analyses. Five cells from each hemisphere were analysed. NEUROLUCIDA® software was used to quantify spine density of selected dendrites as determined by the number of visible spines per 10-μm length of dendrite (at 5000 × magnification). For medium spiny neurones, one third-order (or greater) terminal tip was identified and the total number of visible spines along the length of the dendritic segment (at least 20 μm long) was counted. One dendritic segment was analysed per neurone. Spines were always counted from the last branch point to the terminal tip of the dendrite. For cortical neurones, spines were counted on one third-order tip. No attempt was made to correct for the fact that some spines are obscured from view, so the measure of spine density necessarily underestimates total spine density. Anatomical analysis was conducted blind to treatment condition.

Statistical analyses

All of the results of the statistical tests used are indicated in detail in the figure legends.

Locomotor activity

Differences in scores of total distance travelled were analysed using two-way repeated measures ANOVAs with genotype and time (min) as between- and within-group variables, respectively. Student's *t*-tests for independent samples were used to analyse differences in stereotypy counts between groups.

Prepulse inhibition

Differences in baseline magnitude of startle response were analysed using Student's *t*-test for independent samples. Differences in baseline PPI scores were analysed using two-way repeated measures ANOVAs with genotype and prepulse intensity as between- and within-group variables, respectively. Data obtained from the AMPH experiments were analysed using three-way repeated measures ANOVAs with genotype and treatment as between-group variables and prepulse intensity as the within-group variable. Posthoc analyses of significant interactions were made using ANOVA tests for simple effects. Baseline measures of startle response and PPI obtained in the 'low' and 'high' experiments were combined.

Conditioned place preference

Place preference scores (i.e. difference in time spent in the AMPH- vs. the saline-paired compartment before and after conditioning) were analysed using planned paired *t*-tests with the significance level adjusted for multiple comparisons using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979).

Microdialysis

Data obtained before and after the AMPH challenge were analysed using two-way repeated measures ANOVAs with genotype and time (min) as between- and within-group variables, respectively. Posthoc analyses of significant interactions were made using ANOVA tests for simple effects.

Results

Blunted behavioural activation by AMPH in DCC-deficient male and female mice

We determined whether the phenotype previously reported for *dcc* heterozygous mice is dependent on a specific genetic background and/or sex. We previously reported that adult *dcc* heterozygous (+/–) male mice of a 129Sv/BL6 cross (Fazeli *et al.*, 1997) show a blunted locomotor response to a single AMPH injection of 1.5, 2.5 or 4 mg/kg (Flores *et al.*, 2005). Here, however, we conducted all of the experiments in a pure BL6 strain (Burgess *et al.*, 2006). As previously found, there was no effect of genotype on basal locomotor activity or following a single i.p. injection of saline (Fig. 1a and b). However, when male mice were given a single AMPH injection, +/– mice were significantly less responsive in comparison to their +/+ littermate controls (Fig. 1c).

We then assessed locomotor activity in adult cycling female +/– and +/+ mice and obtained results identical to the data obtained in males; there was no genotype effect on baseline locomotor activity (Fig. 1a) or following an injection of saline (Fig. 1b). However, a blunted locomotor response to AMPH was observed in +/– mice in comparison to +/+ controls (Fig. 1c). Consistent with previous work (Morse *et al.*, 1995), the locomotor activity observed in male and female BL6 mice was very similar.

Finally, both male and female +/– mice, in comparison to +/+, demonstrated significantly fewer stereotypy counts in the AMPH test (Fig. 1d), ruling out the possibility that the genotype effect was caused by drug-induced stereotypy. Together, these results indicate that the *dcc* phenotypic response to AMPH does not depend on genetic strain or gender. See Fig. 1 legend for statistical analysis.

Reduced DCC protects against AMPH -induced sensorimotor gating deficits

Sensorimotor gating, a fundamental form of information processing dependent on proper mesocorticolimbic DA function, is impaired in schizophrenia. It has been shown that single or repeated exposure to stimulant drugs, such as AMPH, produces significant deficits in sensorimotor gating in laboratory animals (Tenn *et al.*, 2005). This effect appears to be mediated by AMPH-induced DA release in the NAcc (Swerdlow *et al.*, 1990). For this reason, performance on tests of sensorimotor gating is widely used to assess deficits in animal models of schizophrenia. Sensorimotor efficacy can be measured by PPI, the phenomenon by which a mild stimulus (prepulse) suppresses the response to another strong startle-eliciting stimulus. Here, we assessed differences in PPI between adult +/– and +/+ mice both at baseline and following a single AMPH injection. No differences in response to startle alone, baseline PPI or habituation to the testing session (data not shown) were observed between +/– and +/+ mice (Fig. 2a and b). In the low-dose AMPH experiment, although +/+ mice appeared to

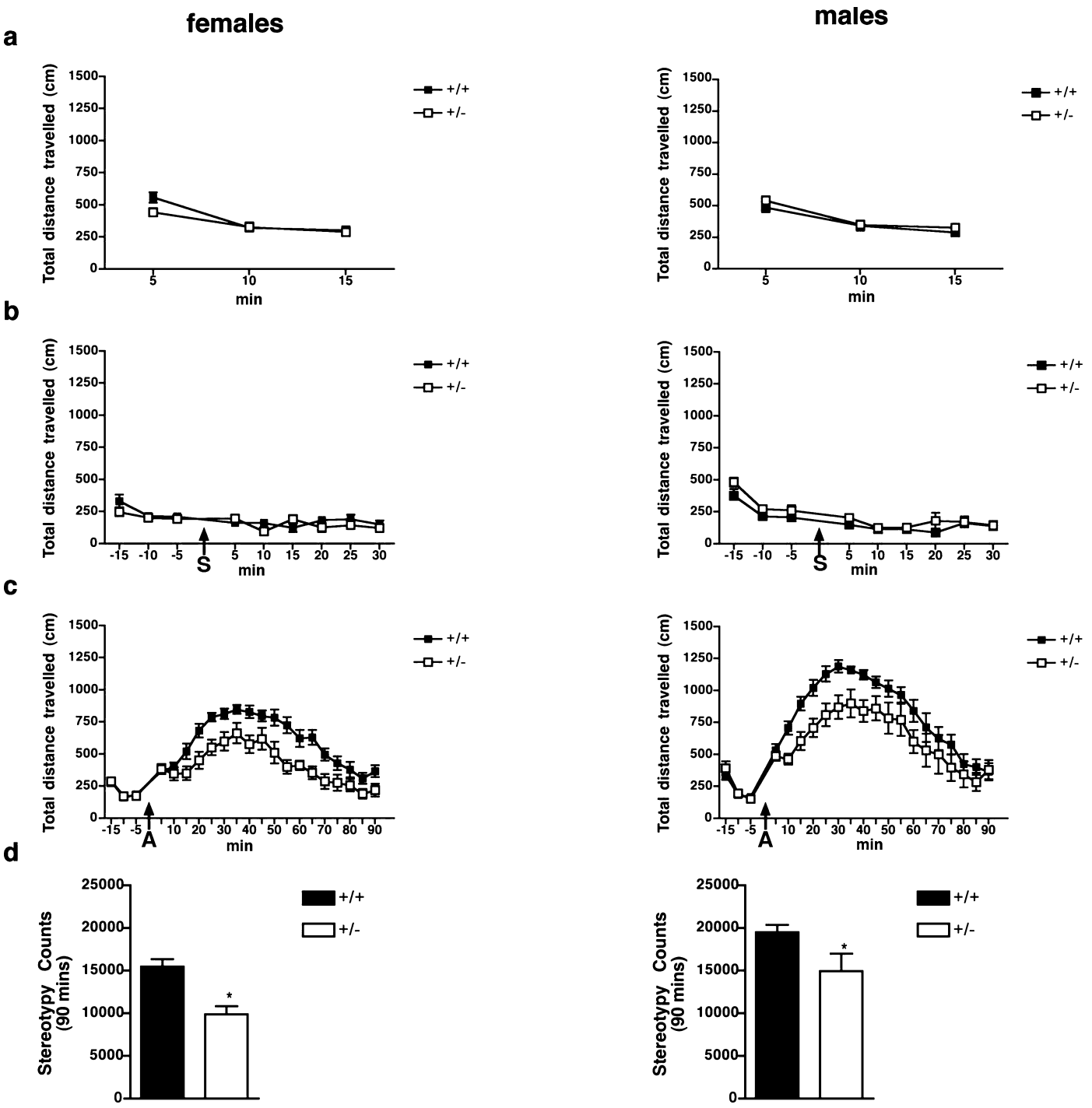

FIG. 1. Adult male ($n = 8$) and female ($n = 9$) *dcc* +/– mice are less sensitive to the locomotor-enhancing effects of amphetamine (AMPH) than +/+ littermates ($n = 11$ and 10). Data points represent total distance (cm) travelled (mean ± SEM). Injection time point is indicated by an arrow. (a) No difference between groups was observed during the habituation period (day 1). (b) On day 2, no difference between +/– and +/+ mice was found following an injection of 0.9% saline at 15 min after habituation. (c) On day 3, the locomotor response to a single injection of AMPH (males, 2.5 mg/kg; females, 2.2 mg/kg, i.p.) was blunted in +/– mice. Repeated measures ANOVA revealed a significant main effect of genotype (males, $F_{1,17} = 7.7$, $P = 0.01$; females, $F_{1,17} = 12.2$, $P = 0.002$). (d) Stereotypy counts following AMPH administration (males, $t_{17} = 2.5$, $^*P = 0.03$; females, $t_{17} = 4.2$, $^*P = 0.0005$). A, AMPH injection; S, saline injection.

exhibit higher PPI scores than +/– mice, statistical analysis revealed no effect of genotype and no interaction between genotype and treatment (see Fig. 2c for details of statistical analysis). However, there was a significant effect of AMPH but only in the +/+ mice. Remarkably, even after doubling the AMPH dose (6.4 mg/kg), +/– mice remained insensitive to AMPH-induced PPI impairments (Fig. 2c). The low baseline percentage of PPI and following AMPH injection is typical for BL6 mice, which exhibit lower PPI and are more sensitive to the behavioural effects of AMPH than other strains (Varty *et al.*, 2001).

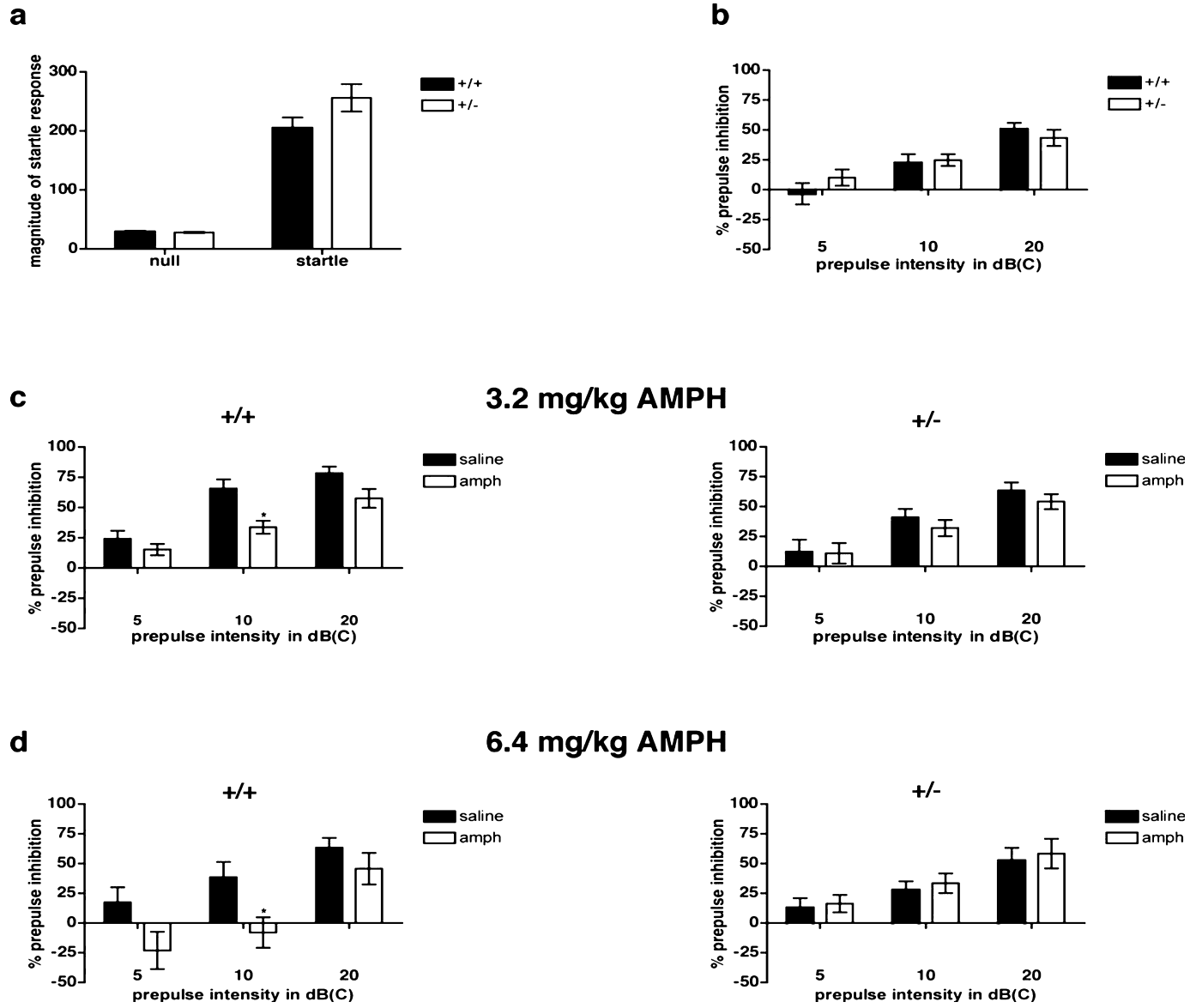

FIG. 2. Adult *dcc* +/– mice are protected against amphetamine (AMPH)-induced deficits in prepulse inhibition (PPI). (a and b) +/– ($n = 28$) and +/+ ($n = 27$) mice showed no difference in magnitude of startle response at baseline ($t_{53} = 1.69$, $P = 0.1$). Repeated measures ANOVA revealed no significant effect of genotype ($F_{1,54} = 0.27$, $P = 0.60$) and no significant interaction ($F_{2,108} = 0.38$, $P = 0.68$). (c) For the low AMPH dose experiment, a three-way ANOVA with genotype and treatment as between-group variables and prepulse intensity as the within-group factor revealed no significant effect of genotype ($F_{1,27} = 2.76$, $P = 0.1$) and no significant interaction ($F_{1,54} = 1.31$, $P = 0.26$) but there was a main effect of treatment ($F_{1,27} = 4.92$, $P = 0.03$). *Student's *t*-test for independent samples revealed a significant reduction in PPI in +/+ mice treated with AMPH ($t_9 = 2.6$; $P = 0.03$). (d) For the high AMPH dose experiment, a three-way ANOVA with genotype and treatment as between-group variables and prepulse intensity as the within-group factor revealed a significant interaction between genotype and treatment ($F_{1,19} = 5.55$, $P = 0.03$). A posthoc ANOVA test for simple effects indicated a significant effect of AMPH in +/+ mice ($F_{1,19} = 8.5$, $P = 0.009$) but not in +/– mice. No differences between genotypes was observed in saline-treated animals ($F_{1,19} = 0.54$, $P = 0.47$). Low-dose experiment: saline +/+, $n = 6$; +/–, $n = 9$; AMPH, +/+, $n = 8$; +/–, $n = 8$. High-dose experiment: saline +/+, $n = 6$; +/–, $n = 6$; AMPH, +/+, $n = 5$; +/–, $n = 6$.

These findings are consistent with the idea that the *dcc* heterozygous phenotype only becomes evident upon AMPH challenge. See Fig. 2 legend for statistical analysis.

Reduced DCC diminishes AMPH-induced reward

Sensitized striatal DA function in schizophrenia has been suggested as a possible explanation for the high comorbidity between schizophrenia and drug abuse (Chambers *et al.*, 2001). Moreover, increased NAcc DA release is known to play a critical role in mediating the rewarding effects of AMPH (Di Chiara *et al.*, 2004). We therefore tested whether *dcc* heterozygous mice would be less sensitive to the rewarding effects of AMPH using CPP.

In the CPP paradigm, animals learn to associate the effects of a drug with a particular environment. Therefore, preference for the drug-paired environment later on can be considered as an index of the rewarding properties of the drug. Sensitivity to the rewarding effects of AMPH were determined in a place preference test conducted 1 day after three consecutive days of conditioning trials, in which AMPH and saline were paired to particular compartments. As previously

shown (Budygin *et al.*, 2004) in a postconditioning test, +/+ mice spent a significantly greater amount of time in the compartment previously paired with 2.2 mg/kg of AMPH than in the one paired with saline. This preference, however, was not observed in +/– mice. When mice were tested with a higher AMPH dose (4.4 mg/kg), a rightward shift in the dose-effect response was observed in the +/– group; both groups exhibited significant preference for the AMPH-associated compartment (Fig. 3). These results indicate that +/– mice have a diminished sensitivity to the incentive properties of AMPH because they fail to show preference for an environment previously paired with an AMPH dose that does induce CPP in wild-type controls. These findings raise the interesting possibility that subtle alterations in the balance of netrin-1 receptors render individuals more or less prone to drug abuse. See Fig. 3 legend for statistical analysis.

Impaired AMPH-induced dopamine release in nucleus accumbens in dcc heterozygotes

AMPH-induced locomotion, CPP and PPI deficits have been shown to be dependent on AMPH-induced DA release in NAcc in rodents (Swerdlow *et al.*, 1990; Sellings & Clarke, 2003). Thus, we predicted that the behavioural phenotype observed in the adult +/– mice would be associated with altered AMPH-induced DA release in this region. Here we assessed NAcc DA function through *in-vivo* microdialysis experiments on freely moving mice during baseline conditions and following a single injection of AMPH (Fig. 4). Because, in the behavioural tests conducted in this study and in our previous study (Flores *et al.*, 2005), we found no differences between genotypes following a single injection of saline, we only conducted microdialysis experiments in amphetamine-treated animals.

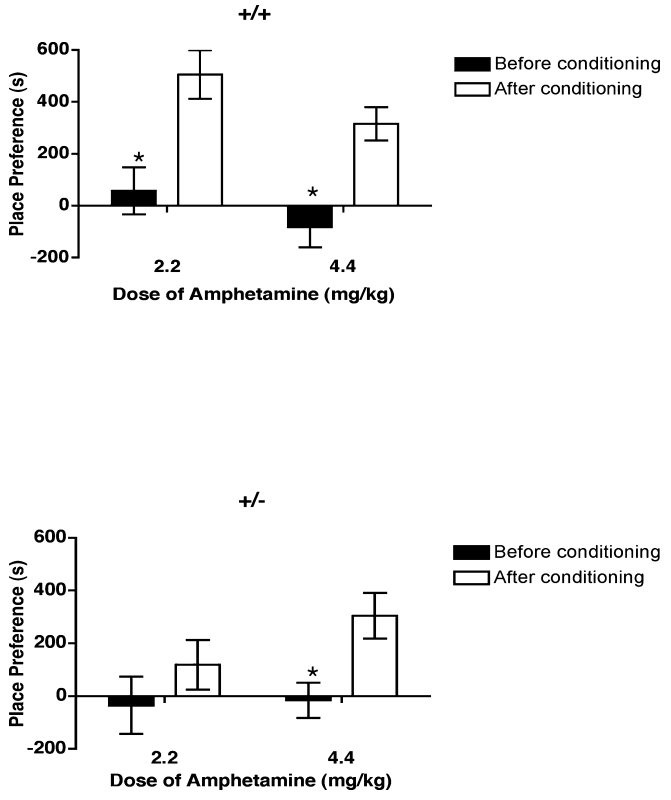

FIG. 3. Preference for the compartment associated with amphetamine (AMPH) in adult *dcc* +/– and +/+ mice ($n = 10$/group). Place preference is expressed as the difference in time spent in the AMPH- vs. the saline-paired compartments before and after conditioning. Planned paired *t*-tests, with the significance level adjusted for multiple comparisons using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979), revealed that although a lower dose of AMPH (2.2 mg/kg) established a place preference for the drug-paired compartment in +/+ mice ($t_9 = 2.884$, $P = 0.018$; adjusted $\alpha = 0.025$), it failed to do so in +/– mice ($t_9 = 1.312$, $P = 0.222$). When mice were given 4.4 mg/kg of AMPH, however, both +/+ ($t_9 = 3.805$, $P = 0.004$; adjusted $\alpha = 0.013$) and +/– ($t_9 = 3.327$, $P = 0.009$; adjusted $\alpha = 0.017$) mice spent more time in AMPH-associated compartments after conditioning than they did before. $^*P < 0.05$.

Baseline levels of extracellular DA did not differ between +/– and +/+ mice. This finding is consistent with the lack of genotype effect observed in baseline locomotor activity and PPI. However, there were considerably lower baseline extracellular concentrations of DOPAC and homovanilic acid in +/– mice, indicating decreased DA activity in this region. These findings concur with the results of our previous study where high-performance liquid chromatography analysis was conducted on whole tissue punches taken from the NAcc of +/+ and +/– mice (Flores *et al.*, 2005).

Importantly, however, and in contrast to the lack of differences in baseline extracellular DA, there was a large effect of genotype on DA release induced by an AMPH challenge. Although AMPH produced a large increase in extracellular DA concentrations in the NAcc of +/+ mice, it produced less than half this effect in +/– mice. It is important to point out that DOPAC concentrations in +/– mice were only slightly reduced by the AMPH challenge because the baseline levels were already very low. See Fig. 4 legend for statistical analysis.

DCC deficiency results in enhanced medial prefrontal cortex dopamine activity

Dopamine activity in the NAcc is highly regulated by DA neurotransmission in the mPFC. In fact, mPFC DA activity has been shown to attenuate mesolimbic DA release (Deutch *et al.*, 1990; Sesack & Pickel, 1992; Ventura *et al.*, 2004). Thus, we hypothesized that the blunted DA response to AMPH observed in the NAcc of +/– mice results from greater DA activity in the mPFC. To this end, we measured DA and DA metabolites in the mPFC of adult +/– and +/+ mice using *in-vivo* microdialysis. In agreement with our hypothesis, +/– mice showed, at the beginning of the sample collection period, significantly higher baseline extracellular levels of mPFC DA and DOPAC, indicating hyperactivity of the mesocortical DA system (Fig. 5). Although homovanilic acid concentrations were also elevated in mPFC of +/– mice, this effect was not statistically significant. These findings are in accordance with our previous results on whole tissue punches (Flores *et al.*, 2005).

There were large differences in the DA response to AMPH (2.5 mg/kg) in the mPFC between +/– and +/+ mice. It can be seen in Fig. 5 that the increase in DA in the mPFC of +/– mice was significantly greater than in +/+ mice. These results show that mPFC DA function is significantly enhanced in +/– mice and suggest that the hyperactivation in this region by AMPH may account for the blunted AMPH-induced DA release in the NAcc and the behavioural effects observed.

The evidence suggests that, in addition to DA, mPFC norepinephrine also plays a role in the effects of AMPH on locomotion, PPI and reward (Blanc *et al.*, 1994; Ventura *et al.*, 2003; Swerdlow *et al.*, 2006). We therefore measured extracellular mPFC norepinephrine concentrations before and after the AMPH challenge. No differences, however, were observed between +/– and +/+ mice at baseline (+/+ mice, 5.4 ± 1.1 pg/10 μL; +/– mice, 5.2 ± 1.2 pg/10 μL) or after

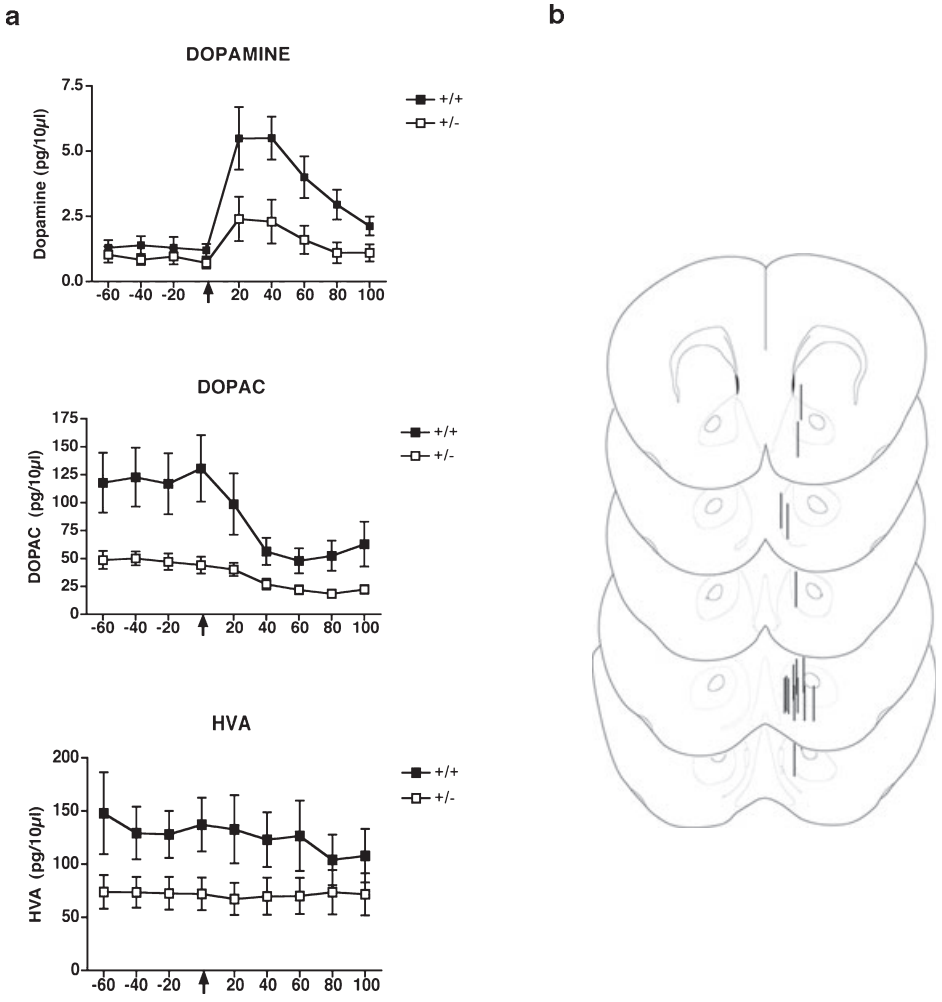

FIG. 4. Extracellular concentrations of dopamine (DA) in the nucleus accumbens of adult *dcc* +/– mice during baseline and following a amphetamine (AMPH) injection. Dialysates were collected through microdialysis probes and analysed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) (mean ± SEM). Samples were collected during baseline and following an injection of AMPH (2.5 mg/kg, i.p. indicated by an arrow). (a) Data obtained before and after the AMPH challenge were analysed using two-way repeated measures ANOVAs, with genotype and time (min) as variables. DA: Although there was no difference in basal levels of DA between +/– and +/+ mice (main effect of genotype: $F_{1,14} = 1.3$, $P = 0.28$; genotype by time interaction: $F_{3,42} = 1$, $P = 0.4$), a significant main effect of genotype in AMPH-induced DA release was revealed ($F_{1,15} = 6.04$, $P = 0.02$) and significant interaction ($F_{4,60} = 4.01$, $P = 0.006$). DA metabolites: Extracellular concentrations of DOPAC at baseline and following AMPH challenge were reduced in +/– as compared with +/+ mice (baseline: main effect of genotype, $F_{1,15} = 6.5$, $P = 0.02$; interaction, $F_{3,45} = 1.7$, $P = 0.16$; AMPH: main effect of genotype, $F_{1,15} = 4.8$, $P = 0.04$; interaction, $F_{4,60} = 3.6$, $P = 0.01$). Extracellular concentrations of HVA at baseline and following AMPH challenge were reduced in +/– as compared with +/+ mice but these effects did not reach statistical significance (baseline: main effect of genotype, $F_{1,16} = 3.7$, $P = 0.07$; AMPH: main effect of genotype, $F_{1,15} = 2.2$, $P = 0.15$; with no significant interactions). (b) Placement of the tip of the probes was verified using Nissl staining; only data from animals with correct probe placement were used in the analysis ($n = 7$–10/group).

the AMPH challenge (+/+ mice, 5.8 ± 0.8 pg/10 µL; +/– mice, 5.9 ± 0.7 pg/10 µL) (data not shown). See Fig. 5 legend for statistical analysis.

Reduced DCC is not associated with compensatory changes in the expression of UNC5H

Netrin-1 is a bifunctional cue that can attract or repel growing axons depending on the balance between 'attractive' and 'repulsive' receptors that these axons express. It is generally accepted that, whereas attraction is mediated by DCC receptors, repulsion occurs through DCC/UNC5H receptor complexes (Manitt & Kennedy, 2002). To determine whether decreased expression of DCC would be associated with compensatory changes in the expression of UNC5H, we conducted western blot analysis on tissue punches excised from the VTA, mPFC and NAcc of adult +/– and +/+ mice. We used a pan-UNC-5 antiserum, which recognizes UNC-5H1, UNC-5H2 and UNC-5H3 homologues (Manitt *et al.*, 2004). As expected, significant decreases in DCC protein levels were found in brains of +/– mice as previously shown (Flores *et al.*, 2005). However, no differences in UNC5H protein expression were observed between the two genotypes (Fig. 6). These results indicate that *dcc* haploinsufficiency indeed alters the balance between DCC and UNC5H protein expression. See Fig. 6 legend for statistical analysis.

Both DCC and UNC5H receptors are expressed by ventral tegmental area dopamine neurones

Our results suggest that regulation of netrin-1 receptor expression leads to functional reorganization of midbrain DA systems. It remains

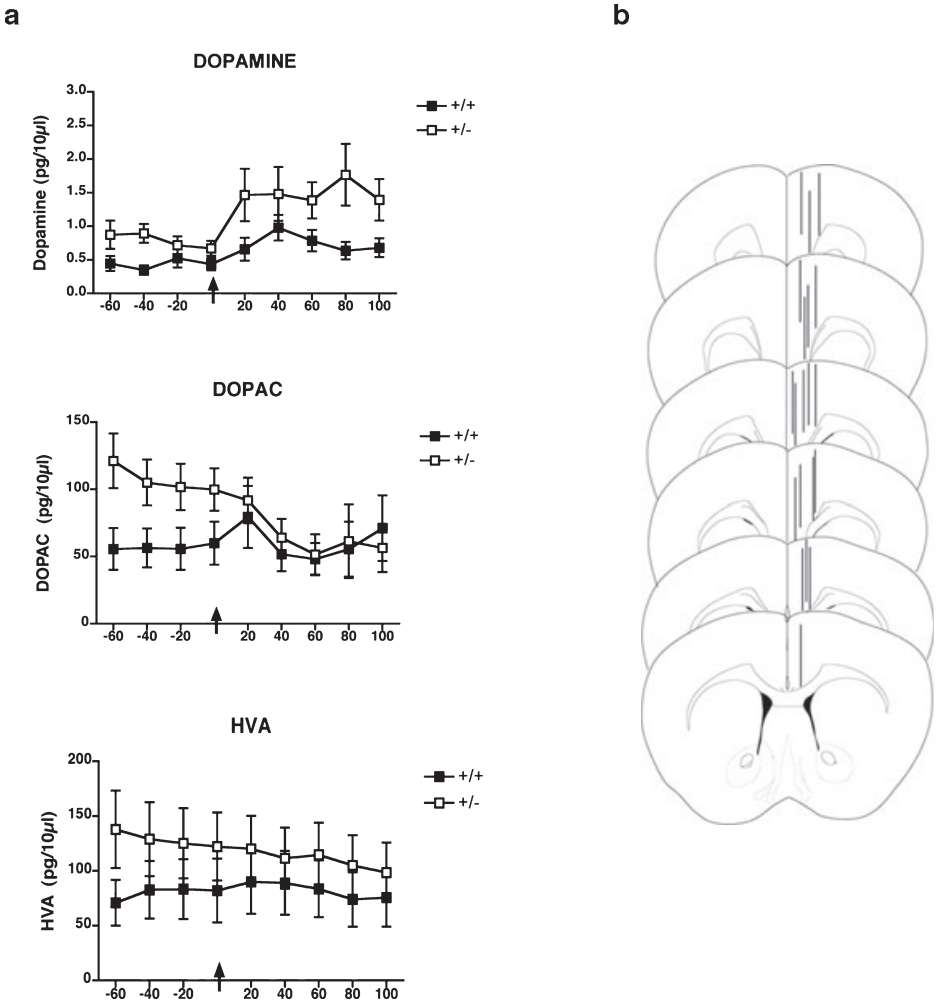

FIG. 5. Extracellular concentrations of dopamine (DA) in the medial prefrontal cortex of adult *dcc* +/– mice during baseline and following a amphetamine (AMPH) injection. Dialysates were collected through microdialysis probes and analysed for extracellular levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) (mean ± SEM). Samples were collected during baseline and following an injection of AMPH (2.5 mg/kg, i.p. indicated by an arrow). (a) Data obtained before and after the AMPH challenge were analysed using two-way repeated measures ANOVAs, with genotype and time (min) as variables. DA: For baseline DA, there was a significant main effect of genotype ($F_{1,17} = 4.1$, $P = 0.05$) and a significant genotype by time interaction ($F_{3,51} = 2.7$, $P = 0.05$). In addition, a significant main effect of genotype in AMPH-induced DA release was revealed ($F_{1,16} = 4.9$, $P = 0.04$) with no significant interaction ($F_{4,64} = 0.9$, $P = 0.47$). DA metabolites: Baseline concentrations of DOPAC were significantly higher in +/– as compared with +/+ mice (main effect of genotype, $F_{1,14} = 4.8$, $P = 0.04$; interaction, $F_{3,42} = 2.5$, $P = 0.07$). Although baseline HVA concentrations were reduced in +/– mice, this effect was not statistically significant ($F_{1,17} = 1.3$, $P = 0.26$; interaction: $F_{3,51} = 2.3$, $P = 0.08$). (b) The placement of exposed tips of microdialysis probes was verified using Nissl staining and is indicated on the coronal plates taken from Paxinos & Franklin (2001). Probe placements of +/– and +/+ groups were evenly distributed across the dorsal/ventral and rostral/caudal axes. Only data from animals with correct probe placement were used in the analysis ($n = 7$–10/group).

to be addressed, however, whether midbrain DA neurones do indeed express both attractive and repulsive receptors. Using double-labelling immunofluroescence, we assessed whether VTA DA neurones express netrin-1 receptors in adult +/– and +/+ mice. We found that both DCC and UNC5H proteins are expressed in VTA neurones of +/– and +/+ mice and that, importantly, both proteins are expressed in TH-positive cells (Fig. 7). In addition, several double-labelled DCC/UNC5H neurones were observed in the VTA, strongly suggesting that both types of receptors are expressed by single DA neurones. Finally, DCC and UNC5H immunolabelling was observed in TH-positive terminals within the mPFC and NAcc (data not shown). These results, although in agreement with those reported previously on DCC expression in the adult mouse (Osborne *et al.*, 2005), are to our knowledge the first to demonstrate UNC5H expression in adult midbrain DA neurones.

dcc haplo-insufficiency leads to changes in neuronal structure in the medial prefrontal cortex

Reduced DCC leads to substantial changes in mesocorticolimbic DA function and DA-dependent behaviours in the adult. However, the processes underlying this effect remain to be determined. As a first step toward addressing this question, we assessed whether reduced DCC results in enduring reorganization of mesocorticolimbic DA circuitry. Because the vast majority of synaptic inputs onto neurones are on dendritic spines, we analysed differences in dendritic spine density in NAcc and mPFC regions that receive robust DA innervation (Fallon & Loughlin, 1987) in Golgi-Cox-processed brains of adult +/– and +/+ mice. In NAcc medium spiny neurones, no difference in dendritic spine density was observed between +/– and +/+ mice (Fig. 8a). Remarkably, however, in layer V mPFC neurones, +/– mice show a large and significant reduction (~ 40%) in dendritic spine

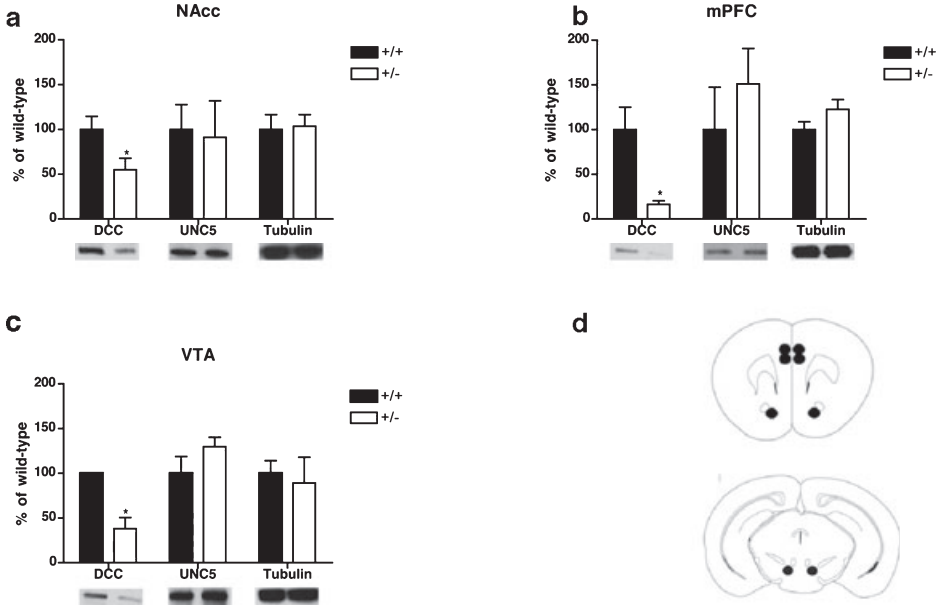

FIG. 6. Expression of netrin-1 receptors in adult +/– and +/+ mice in baseline conditions. (a-c) Optical density data were converted to percent of the +/+ group. Student's *t*-tests for independent samples revealed significantly lowered expression of DCC in +/– mice ($n = 4$–8/group) in (a) the nucleus accumbens (NAcc) ($t_7 = 2.5$, $^*P = 0.05$), (b) medial prefrontal cortex (mPFC) ($t_{15} = 3.6$, $P = 0.003$) and (c) ventral tegmental area (VTA) ($t_6 = 3.7$, $P = 0.04$). No differences between groups were found in UNC5H or β-tubulin expression. Representative examples of western blots for +/– and +/+ mice are shown below the graphs. (d) Representative locations of bilateral tissue punches of the mPFC, NAcc (top) and VTA (bottom) used for western blot analysis.

density, in comparison to the +/+ group (Fig. 8b). This reduction appeared to be specific to pyramidal neurones localized in a cortical region highly innervated by DA neurones because no differences between groups were observed in layer III pyramidal cells. Our findings are therefore consistent with the idea that alterations in levels of netrin-1 receptors during development may lead to reorganization of synaptic connectivity of mesocortical DA system. See Fig. 8 legend for statistical analysis.

Discussion

In this study we show that adult male and female mice that develop with decreased levels of the netrin-1 receptor DCC, a developmental protein involved in directing growing axons toward appropriate targets, have elevated basal as well as AMPH-induced extracellular DA concentrations in the mPFC but decreased AMPH-induced DA activity in the NAcc. Correspondingly, these *dcc* heterozygous mice exhibit blunted locomotor activation in response to a single injection of AMPH, are resistant to AMPH-induced deficits in sensorimotor gating and are less sensitive to the rewarding properties of AMPH. Together, these findings show that *dcc* haplo-insufficiency results in a phenotype that is opposite to that observed in developmental animal models of schizophrenia (Boksa, 2004; Harrison & Weinberger, 2005; Chen *et al.*, 2006; Ross *et al.*, 2006).

Numerous pharmacological, stress and lesion experiments show that DA function in the NAcc and mPFC can be regulated differently and that, in fact, DA function in the mPFC has an inhibitory control over DA activity in the NAcc. For instance, selective lesions of the DA input to the mPFC have been shown to result in increased DA release in the NAcc via disinhibition of cortical glutamatergic efferents (Deutch *et al.*, 1990; Sesack & Pickel, 1992; Ventura *et al.*, 2004). Conversely, stimulation of DA receptors in the mPFC decreases DA activity in the NAcc (Vezina *et al.*, 1991; Banks & Gratton, 1995; Thompson & Moss, 1995; Doherty & Gratton, 1996). Thus, the reduced NAcc DA activity observed in *dcc* heterozygous mice at baseline and, importantly, following the AMPH challenge is likely to result from hyperactivity of DA in the mPFC.

Ventura *et al.* (2004) showed that BL6 mice exhibit a smaller increase in extracellular concentrations of mPFC DA following an AMPH challenge in comparison to DBA/2J mice. They also showed that this smaller increase accounts for the greater AMPH-induced DA release in the NAcc observed in the BL6 background; selective mPFC DA depletion in DBA/2J mice abolished differences in DA release in NAcc between the two strains. Similar to their observations, we found a small response to AMPH in wild-type BL6 mice used in this study. Interestingly, *dcc* haplo-insufficiency reverses this poor mPFC response to AMPH, resulting, in turn, in significantly diminished AMPH-induced DA release in the NAcc. Behaviourally, these DCC-deficient mice show blunted AMPH-induced locomotion and reward (as measured in the CPP paradigm) and are protected against AMPH-induced deficits in sensorimotor gating. This pattern of results is consistent with the fact that many of the behavioural effects of AMPH are dependent on AMPH-induced DA release in the NAcc (Swerdlow *et al.*, 1990; Sellings & Clarke, 2003).

A complete understanding of the mechanisms underlying the effects of *dcc* haplo-insufficiency on baseline as well as on AMPH-induced alterations in extracellular concentrations of DA and DA metabolites in the NAcc and mPFC is yet to be determined. Changes in the expression and/or activity of proteins involved in the regulation of DA synthesis, metabolism and clearance are likely to be involved. However, the findings from the present and our previous study (Flores *et al.*, 2005) suggest that the increases in DA activity in the mPFC could originate from anatomical alterations in this region, namely, increased DA fiber ingrowth. In fact, as we showed in our previous study, there is an increase in basal levels of TH expression in the mPFC of adult *dcc* heterozygous mice without a corresponding increase in dopamine-beta-hydroxylase expression. This increase,

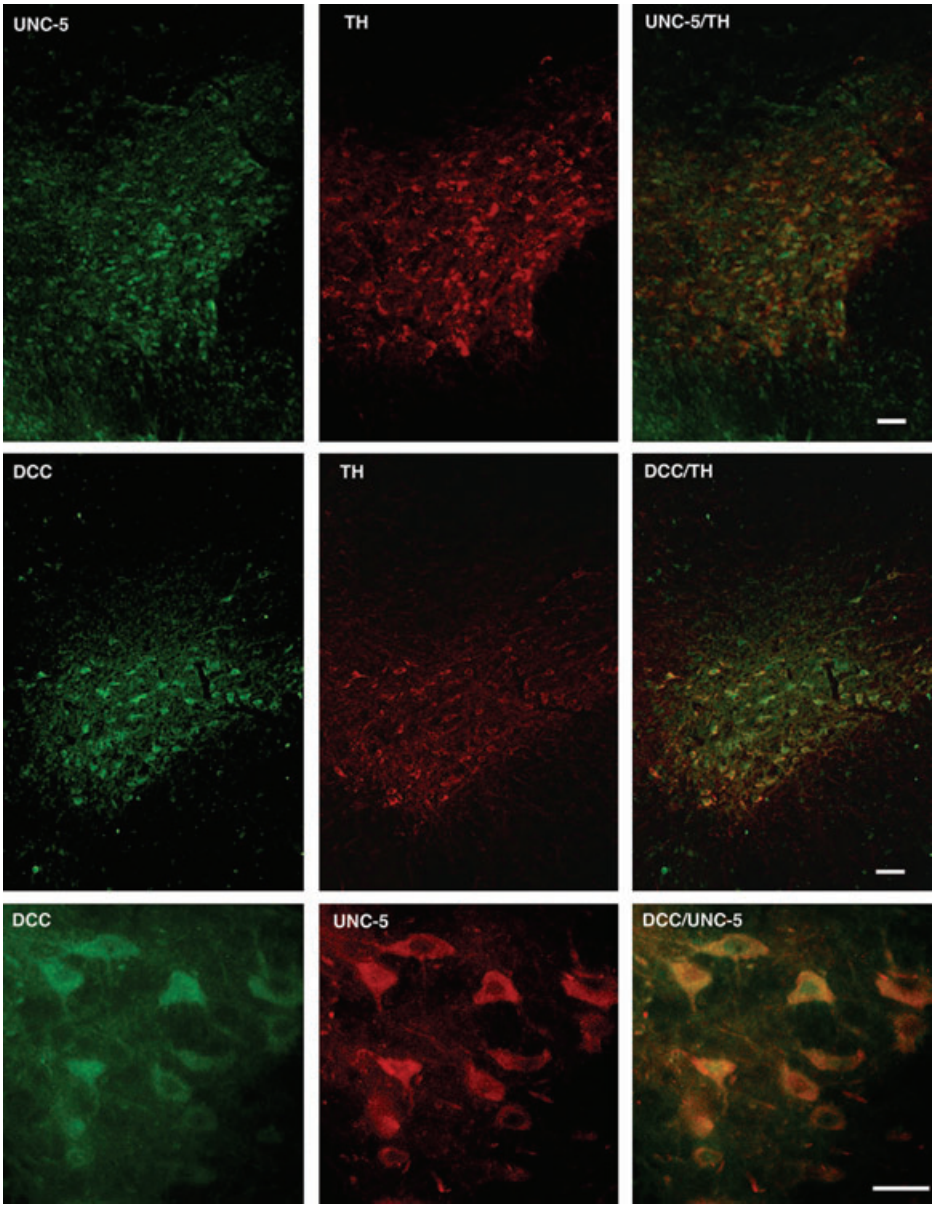

FIG. 7. Localization of netrin-1 receptors in the ventral tegmental area (VTA) of adult +/+ mice. Digitized images of coronal VTA sections from +/+ mice under baseline conditions. Both DCC- and UNC5H-immunopositive cells were visualized in the VTA. Most DCC- and UNC5H-positive cells were also tyrosine hydroxylase (TH) immunoreactive. The same was observed in brains of adult +/– mice. In addition, a double-labelling experiment revealed many VTA neurones coexpressing DCC and UNC5H. Scale bars, 200 μm.

which was not observed in the NAcc or dorsal striatum, suggests axonal sprouting of DA terminals in the mPFC. This interpretation is further justified by the fact that DCC-deficient mice have a small reduction (∼20%) in TH-positive neurones in the VTA and substantia nigra (Flores *et al.*, 2005).

It is important to note that, in addition to DA, norepinephrine function in the mPFC cortex has also been shown to be a critical regulator of the effects of AMPH on DA release in the NAcc and on behaviour (Blanc *et al.*, 1994; Ventura *et al.*, 2003; Swerdlow *et al.*, 2006). However, consistent with the lack of changes in dopamine-beta-hydroxylase expression, our present results from the microdialysis studies revealed no differences in extracellular norepinephrine concentrations at baseline or after an AMPH challenge in the mPFC. This lack of difference suggests that the higher level of DA in the mPFC may, in itself, account for the adult phenotype described here. Nonetheless, changes in the function of other neurotransmitters known to directly or indirectly influence NAcc DA function, such as glutamate (Grace *et al.*, 2007), cannot be ruled out. Furthermore, aberrations in neuronal circuits within brain regions that innervate the mesocorticolimbic DA system, such as the hippocampus, have been shown in the developing brain of *dcc* homozygotes (Barallobre *et al.*, 2000). Although it is not known whether these differences are exhibited by heterozygous mice and, importantly, whether they are present in the adult, they could contribute to the observed phenotype. These possibilities are currently being examined in our laboratory.

Ultimately the question to be addressed is how does reduced DCC during development produce differential effects on DA transmission

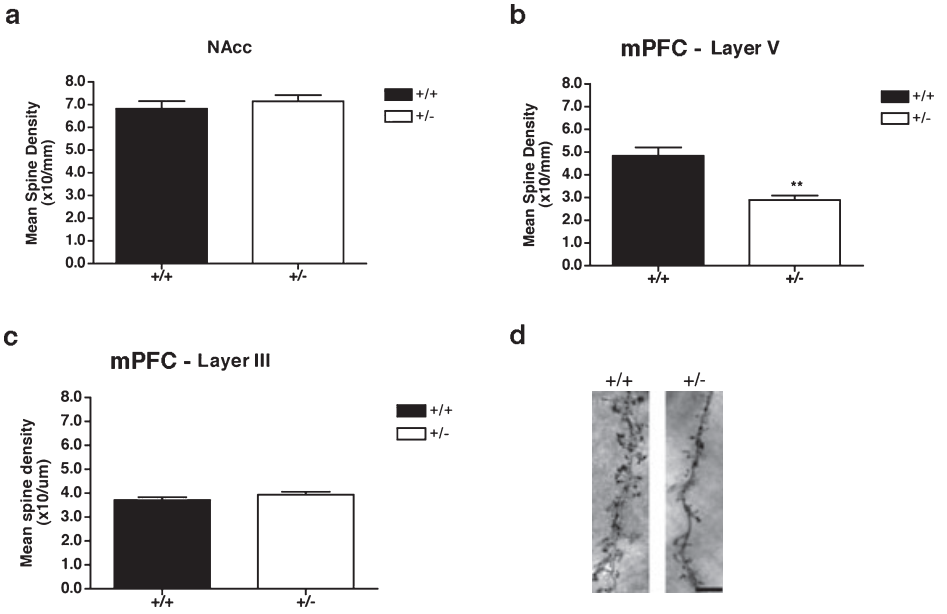

FIG. 8. Dendritic spine density in Golgi-Cox-stained nucleus accumbens (NAcc) medium spiny neurones and medial prefrontal cortex (mPFC) pyramidal neurones of adult *dcc* +/+ and +/– mice (n = 4–6/group). Data points represent group means (± SEM). (a) No difference was observed in NAcc medium spiny neurones between groups. (b) A significant ~40% reduction in dendritic spine density was observed in layer V mPFC pyramidal neurones of *dcc* +/– mice as compared with +/+ littermates (Student's t-tests for independent samples, $t_{10} = 6.477$, $^{**}P = 0.00007$). (c) No difference between groups was observed in layer III mPFC pyramidal neurones. (d) Photomicrograph illustrating representative Golgi-Cox-impregnated dendrites of mPFC pyramidal neurones in the basilar field of layer V of *dcc* +/+ (left) and +/– (right) mice. Scale bar, 5 μm.

and function in the mPFC and NAcc? As mentioned before, it is generally accepted that, whereas attraction is mediated by DCC receptors, repulsion occurs through DCC/UNC5H receptor complexes (Hong *et al.*, 1999; Williams *et al.*, 2003; Bouchard *et al.*, 2004). Thus, subtle changes in the expression of one of these receptors during development should lead to alterations in neuronal connectivity between very specific groups of neurones. Although the mPFC and NAcc components of the mesocorticolimbic DA system originate in the VTA, they have distinctive anatomical and functional characteristics (Le Moal & Simon, 1991; Knable & Weinberger, 1997; Tam & Roth, 1997; Carr & Sesack, 2000). It is likely therefore that variations in DCC alter the organization of one component of this system, which in turn induces functional changes in the other component. Importantly, our results from the western blot analysis show no 'compensatory' decrease in UNC5H protein expression, suggesting that *dcc* haplo-insufficiency does favour UNC5H function.

On the basis of our findings, we hypothesize that reduced DCC leads to selective reorganization of mPFC DA circuitry, which in turn affects DA function in the NAcc. If this is the case, mPFC neurones that receive robust DA innervation would be likely to exhibit alterations in synaptic connectivity (Fallon & Loughlin, 1987). Because the vast majority of synaptic inputs onto neurones are on dendritic spines (Harris & Kater, 1994), we assessed dendritic spine density in neurones within layer V of the mPFC, which receives rich DA innervation, as well as in the NAcc. A large and significant reduction in dendritic spine density was observed in layer V mPFC pyramidal neurones of DCC-deficient mice. In contrast, there was no difference between genotypes in dendritic spine density in NAcc medium spiny neurones. Indeed, the reduced dendritic spine density observed in the mPFC was specific to pyramidal neurones localized to a region highly innervated by DA neurones; spine density of layer III pyramidal cells was not altered in *dcc* heterozygous mice. Intriguingly, the decreases in dendritic spine density found in postmortem brain of schizophrenic patients are observed in pyramidal neurones of layer III (Glantz & Lewis, 2000).

Several studies have shown that changes in dendritic spine density determined using Golgi-Cox staining are accompanied by corresponding changes in the number of synapses per neurones assessed with electron microscopy (Woolley *et al.*, 1990; Woolley & McEwen, 1992; Kolb *et al.*, 1998). For this reason, the alterations in dendritic spine density detected in Golgi-Cox-stained material in the present study can be considered as evidence for changes in patterns of synaptic connectivity (Harris & Kater, 1994). Furthermore, because the number of dendritic spines is inversely associated with synaptic activity (Kirov & Harris, 1999), the reduction in dendritic spine density in layer V mPFC is likely to be a compensatory response to increased DA function in this region. This is consistent with evidence showing that DA neurones in mPFC form synapses onto GABA interneurones (Sesack *et al.*, 1995), which, in turn, regulate the activity of pyramidal cells. Our interpretation that reorganization of mesocortical DA circuitry is the critical determinant of the phenotype observed in the adult *dcc* heterozygotes is also consistent with the facts that (i) the ingrowth of DA terminals into the mPFC is a slow process that continues until early adulthood and therefore has an increased vulnerability for significant structural plasticity (Benes *et al.*, 2000); (ii) adult *dcc* heterozygous mice have increased basal TH protein expression in mPFC but not in NAcc or dorsal striatum (Flores *et al.*, 2005); and (iii) higher levels of DA in the mPFC protect against AMPH-induced DA release in the NAcc and associated behavioural effects, such as deficits in sensorimotor gating. Within this context, it is important to note that the mPFC-NAcc projection arises from layer V pyramidal neurones (Brog *et al.*, 1993; Carr *et al.*, 1999).

To conclude, increasing evidence indicates that subtle disruptions to the normal course of brain development result in increased predisposition to schizophrenia and related disorders later on in life (Weinberger, 1987; Lewis & Gonzalez-Burgos, 2000). Here we show that variations in levels of the netrin-1 receptor DCC during development

may tip the balance between over- and under-mPFC DA influence on NAcc DA function in the adult. When the balance is in favour of over-mPFC influence, the resulting phenotype appears to be protected against the development of behavioural abnormalities, such as those associated with schizophrenia-like symptoms (Seamans & Yang, 2004). These findings add credence to our hypothesis that altered netrin-1 receptor function is a common mechanism by which the diverse perinatal factors could exert their specific enduring effects on brain function and behaviour, rendering individuals more or less vulnerable to psychopathology. Furthermore, they suggest that changes in the balance of netrin-1 receptors may render individuals more or less vulnerable to attention deficits induced by stimulant drugs and less prone to drug abuse. Finally, much speculation has being given to the processes and molecular players involved in the specification and establishment of mesocorticolimbic DA connectivity (Smidt & Burbach, 2007). Our findings suggest that DCC is a critical determinant in the organization of the wiring of this system.

Acknowledgements

We thank S. Ackerman (The Jackson Laboratory) for the original *dcc* heterozygous breeders, T. Pawson (University of Toronto) for the pan-UNC-5 antiserum, G. Luheshi for critical reading of the manuscript, T. Stroh and C. Manitt for their help with the neuroanatomical experiments, and C. Himmelman, X. Wen and Z. Speed for their excellent technical assistance. This work was funded by the Canadian Institute for Health Research (C.F. and J.S.), the Natural Science and Engineering Research Council of Canada (C.F., A.A., B.K. and A.G.), the Canada Research Chairs Program (A.A.) and the Fonds de la Recherche en Santé du Québec (C.F.).

Abbreviations

AMPH, amphetamine; CPP, conditioned place preference; DA, dopamine; DCC, deleted in colorectal cancer; DOPAC, 3,4-dihydroxyphenylacetic acid; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens; PPI, prepulse inhibition; TH, tyrosine hydroxylase; UNC5H, UNC-5 homologues; VTA, ventral tegmental area.

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