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Netrin-1 receptor deficiency protects against psychostimulant-induced behaviours in mice

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Abstract

Psychostimulant drugs, such as cocaine and amphetamine, increase extracellular dopamine in certain brain regions, including those that make up the mesolimbic dopamine system. Dysfunction of this system is implicated in drug addiction. The development of the mesolimbic dopamine system involves netrins, a group of secreted proteins that guide growing axons to their targets. Mice that develop with reduced expression of deleted in colorectal cancer, a netrin-1 receptor, exhibit abnormal dopamine release in response to psychostimulant drugs. Furthermore, these mice show reduced amphetamine-induced locomotor activation when compared to wild-type mice. In this study, our objective is to further examine the drug-induced behaviours of DCC deficient mice. We compared adult DCC deficient and wild-type mice using two behavioural tests. First, we examined the locomotor response of these mice to cocaine. Second, using conditioned place preference, we assessed the rewarding effects of amphetamine. DCC deficient mice showed reduced cocaine-induced locomotor activation and diminished amphetamine-induced reward when compared to wild-type mice. Taken together, this study suggests that DCC deficiency can protect against certain psychostimulant-induced behaviours.

Keywords

DA: *dopamine*
DCC: *deleted in colorectal cancer*
UNC-5: *uncoordinated-5 protein homologue*
TH: *tyrosine hydroxylase*
Nacc: *nucleus accumbens*

Introduction

Psychostimulant drugs, including amphetamine and cocaine, are highly addictive and prone to abuse. Many of the effects of these drugs on humans and rodents are mediated by alterations in dopamine (DA) signaling in the brain. One such change is an acute increase of extracellular DA in the mesolimbic DA system, which is involved in reward and motivation (Ritz and Kuhar, 1993). Activation of this system results in drug-induced behaviours such as locomotor activation and compulsive reward seeking. These behaviours are stereotypical and allow for an operational characterization of addiction that can be studied using laboratory animals (Bozarth, 1986). Sensitization of these behaviours is a characteristic of an addicted state resulting from repeated drug exposure. Importantly, sensitization to the stimulatory effects of psychostimulants is accompanied by changes in the neuronal circuitry of the mesolimbic DA system (Robinson & Kolb, 1999; 2004). Therefore, knowledge of the development of mesolimbic DA circuitry may yield useful information for understanding how this system is altered by psychostimulant drugs.

During brain development, neuronal organization of the mesolimbic DA system relies on both environmental and genetic factors. Alterations to any of these factors can lead to variations in development, connectivity, and neurotransmission of dopaminergic neurons (Lipska et al., 1993; Riddle & Pollock, 2003; Ventura, 2004). Even subtle variations in DA circuitry during development can alter the amount of DA released in the nucleus accumbens (NAcc), the terminal region of mesolimbic DA projections, in response to psychostimulant drugs (Brake, 2004; Di Chiara et al., 2004). This leads to individual variation in the behavioural effects of psychostimulant drugs. However, little is known about what developmental changes can lead to alterations in DA function and to variable responses to psychostimulant drugs. One possibility is that environmental and genetic factors alter the number of growing DA axons that successfully reach their appropriate synaptic targets, leading to variation in dopaminergic circuitry and hence, functioning of the system.

Though the mechanisms underlying the organization of the mesolimbic DA system are poorly understood, recent evidence suggests a role for the netrin family of guidance molecules in the development of this system (Livesey et al., 1997; Lin et al., 2005; Grant et al., 2007). The netrin family, including the mammalian netrin-1, guides growing axons to their appropriate synaptic targets (Dickson, 2002; Manitt & Kennedy, 2002). The effect of netrin-1 is bidirectional; it may attract or repel axons depending on the variety of netrin-1 receptors expressed on the cell surface of the neurite (Barallobre et al., 2005). Binding of netrin-1 to the receptor DCC (deleted in colorectal cancer) causes DCC multimerization, which acts as an attractive signal stimulating neurite growth towards the source of netrin-1 (Stein et al., 2001). If both DCC and another netrin-1 receptor, UNC-5 (uncoordinated-5 protein homologue), are present on the cell surface, binding of netrin-1 results in DCC-UNC-5 heterodimerization, which signals repulsion (Hong et al., 1999). It is therefore postulated that the direction of neurite growth is determined by the relative concentrations of DCC and UNC-5 present on the cell surface. Studies of vertebrate netrin-1 signaling and homologous signaling pathways in C. elegans and D. melanogaster have demonstrated that this signaling pathway plays a fundamental role in nervous system development (Hiramoto, 2000; Barallobre et al., 2005). Interestingly, DCC is highly expressed by midbrain DA neurons during development and in adulthood (Livesey and Hunt, 1997; Lin et al., 2005; Osborne et al., 2005; Grant et al., 2007). UNC-5 is also expressed by these DA neurons in adulthood; however whether they express UNC-5 during development is unknown (Grant et al., 2007). Together, these findings suggest that netrin-1 and its receptors are important for the development and maintenance of the mesolimbic DA system.

To investigate this theory, we used dcc heterozygous knockout mice (as dcc homozygous knockout mice die at birth), which develop with reduced DCC expression (Flores et al., 2005). Though one could hypothesize a compensatory change in UNC-5 expression in response to reduced DCC expression, no such compensatory change exists in adults (Grant et al., 2007). Interestingly, dcc heterozygous mice have reduced DA activity in the NAcc, both at baseline and in response to systemic amphetamine exposure (Grant et al., 2007). In addition, they show altered amphetamine-induced behavioural phenotypes when compared to wild-type

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controls: blunted locomotor activity, an absence of sensitization to this effect after repeated drug exposure, and an absence of sensorimotor gating deficits (Flores et al., 2005; Grant et al., 2007). Drug-free dcc heterozygous animals do not differ from wild-type animals in behavioural testing.

These studies show that netrin-1 signaling plays a role in DA function in the adult mouse. The data also suggests that reduced dcc expression can protect against behavioural effects induced by psychostimulant drugs. In the present study, we aim to further examine the abnormal behavioural responses of dcc heterozygous mice in response to psychostimulant drugs. Using a conditioned place preference paradigm, we assessed cocaine-induced locomotor response and amphetamine-induced reward seeking in both adult dcc heterozygous and wild-type mice. Our results demonstrate that the behavioural phenotype observed in dcc heterozygous mice is not amphetamine-specific. We also demonstrate reduced reward seeking in dcc heterozygous mice.

Methods

Animals

Adult male and cycling female dcc heterozygous C57BL6 mice and wild-type littermates were used for all behavioural experiments. They were obtained from Dr. Suzanne Ackerman (The Jackson Laboratory) and bred at the Douglas Mental Health University Institute animal facility. Pups were weaned at post-natal day 25 and housed with same-sex littermates. Mice were maintained on a constant 12 hour light-dark cycle with ad libitum access to food and water. Only mice between 10 and 13 weeks old were used for behavioural testing. Experiments were controlled for genotype, chamber assignment, and treatment, and were conducted during

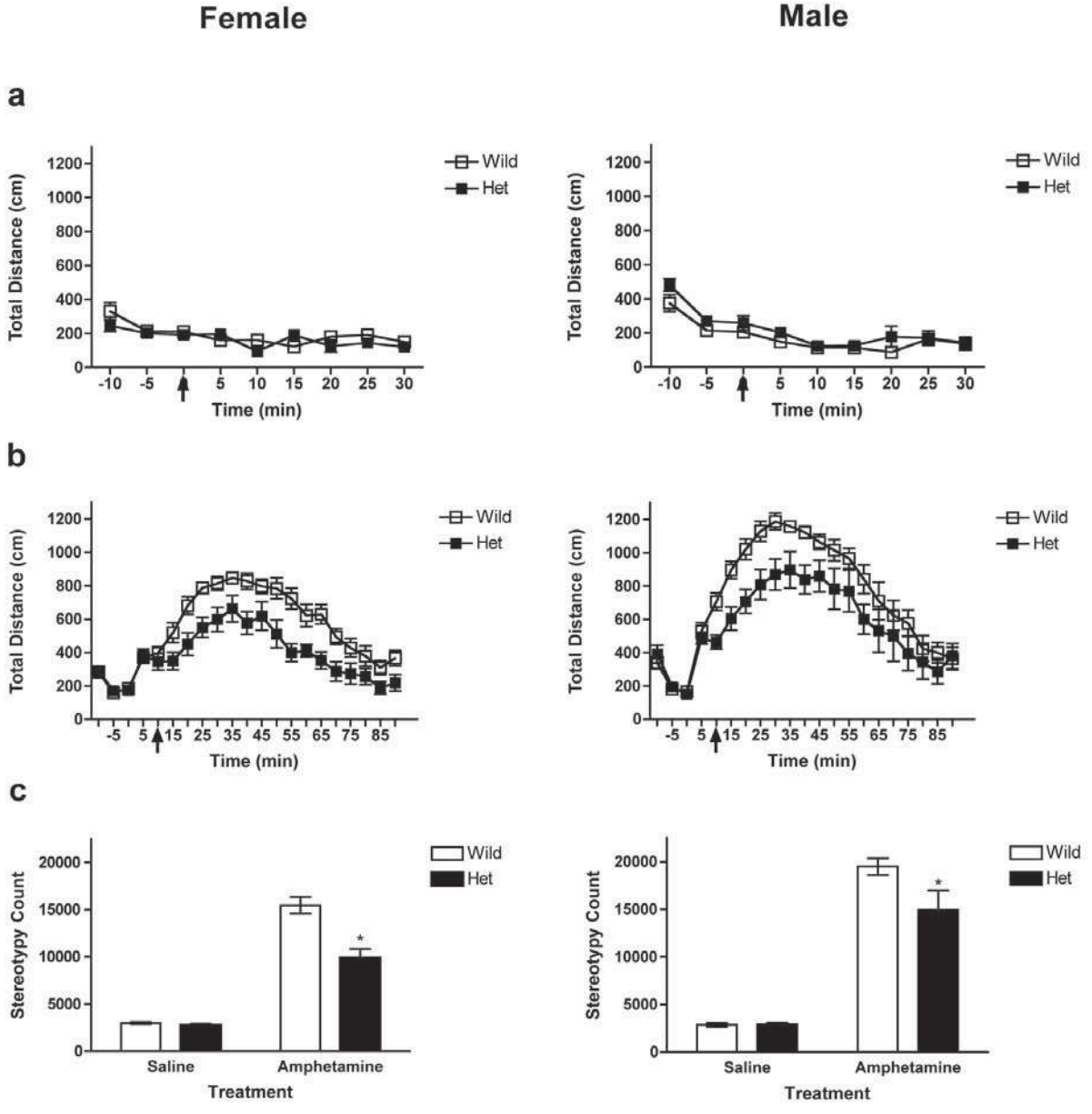

Figure 1: Locomotor response of adult male and female dcc heterozygous mice to an acute injection of amphetamine. Data points represent mean total distance traveled (±standard error means) during a five minute period. Times of injection, at 0 min, are indicated by arrows. (a) No difference in locomotion was seen between dcc heterozygous (Het) and wild-type (Wild) mice following a single injection of 0.9% saline. (b) Following an injection of amphetamine, locomotor activity was blunted in male and female dcc heterozygous mice compared to their wild-type littermates. (c) Stereotypy counts following amphetamine injection, but not a saline injection, were significantly reduced in dcc heterozygous mice compared to wild-type mice.

the light period of the cycle. Only drug-naïve animals were used in this study and separate animals were used for each experiment. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care, the Animal Care Committee of the Douglas Mental Health University Institute, and the Concordia University Animal Research Ethics Committee.

Psychostimulant Drugs

d-Amphetamine sulphate (amphetamine; Sigma-Aldrich, Oakville, Ontario, Canada) and cocaine hydrochloride (cocaine; Medisca, Montréal, Québec, Canada) were dissolved in 0.9% saline. All solutions were administered via intra-peritoneal injections.

Locomotor Activity

Apparatus : The locomotor chambers (AccuScan Instruments, Columbus, Ohio, United States) were made of acrylic and modified for use with mice. Activity-detecting infrared sensors were located in one row on the front and back and two rows on the sides. Data was collected using VersaMax Software version 4.0 (AccuScan Instruments). Locomotor activity was expressed as distance traveled as calculated by the VersaMax system using the spacing between infrared beams. Stereotypy was calculated as the number of repeated breaks of the same infrared beam or series of beams.

Procedure : On the first day the mice were habituated for 15 minutes in the locomotor chambers. On the second day, the mice were habituated for 15 minutes in the locomotor chambers, then given an injection of saline and returned to the locomotor chambers for 30 minutes. On the third day, the mice were again habituated for 15 minutes, then injected with either amphetamine or cocaine and subsequently returned to the locomotor chambers for 90 minutes.

Experiments : In the first experiment male mice were injected with amphetamine at a dose of 2.5 mg/kg.This dose was based on a previous study comparing the amphetamine dose-response of adult dcc heterozygous and wild-type mice (Flores et al., 2005). In the second experiment we used female mice with the dose of amphetamine adjusted for females (2.2 mg/kg) to produce equivalent drug concentrations in the brain (Becker et al., 1982). The remaining two experiments used cocaine at doses of 10.0 mg/kg and 20.0 mg/kg and were conducted in female mice. Since the stimulatory effect of cocaine peaks within 10 to 20 minutes post-injection, we used only the first 30 minutes of collected data to measure the effects of cocaine on locomotion and stereotypy (Tilley et al., 2007).

Conditioned Place Preference

Apparatus : The 3-chambered conditioned place preference apparatus (model ENV-013, MED Associates Inc., Georgia, Vermont, United States) were made of acrylic and modified for use with mice. Infrared sensors on the front and back of each compartment were used to detect activity. MED-PsC software (MED Associates Inc.) used breaks in the infrared beams to record the location of the mouse. All three compartments had distinctive visual and tactile cues, creating three different environments. The two side compartments were of equal size, and cues were balanced such that the mice exhibited no pre-

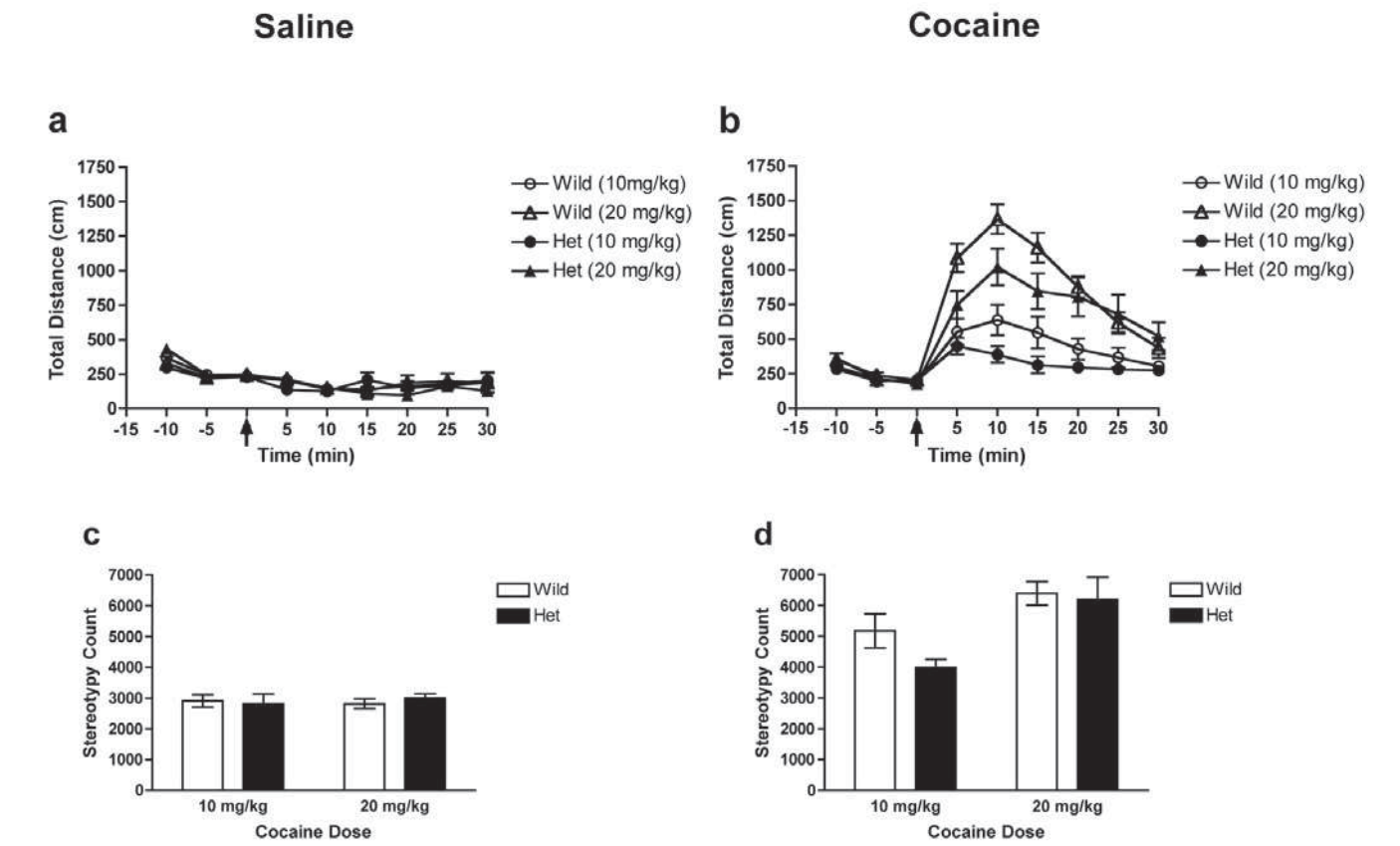

Figure 2: Locomotor response of adult female dcc heterozygous and wild-type mice to injections of cocaine. Data points represent mean total distance traveled (±standard error means) during a five minute period. Times of injection, at 0 min, are indicated by arrows. (a) No difference in locomotion was observed between dcc heterozygous mice (Het) and wild-type mice (Wild) following a single injection of 0.9% saline. (b) Following an injection of cocaine at a dose of 10 mg/kg or 20 mg/kg, dcc heterozygous mice showed significantly reduced locomotor activity compared to wild-type mice. (c) (d) Stereotypy counts following saline and cocaine injections were not significantly different between dcc heterozygous and wild-type mice.

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conditioning preference for either side. The middle compartment was much smaller and used solely as a neutral insertion point on pre-conditioning and post-conditioning days.

Procedure : Testing consisted of three phases: pre-conditioning (day 1), conditioning (days 2, 3, and 4), and post-conditioning (day 5). On day 1, the mice were placed in the middle compartment with free access to all compartments for 30 minutes. This was conducted in four squads between 11:00 am and 1:30 pm, midway between the saline and amphetamine pairings on days 2, 3 and 4. Time spent in each compartment was recorded as the duration of time, in seconds, that an infrared beam in a compartment was broken. For conditioning, mice were randomly chosen to receive amphetamine in one of the side compartments (paired compartment) and saline in the other (unpaired compartment). Compartment-treatment association was balanced such that no particular environment was associated with one treatment more than the other. Likewise, equal treatment was given to both genotypes. Between 9:00 am and 11:30 am on days 2, 3, and 4, mice received saline and were confined to the unpaired compartment for 30 minutes. Four hours later the mice received amphetamine and were confined to the paired compartment for 30 minutes. On day 5, between 11:00 am and 1:30 pm, the mice were again placed in the middle compartment with free access to all compartments for 30 minutes.Time spent in each compartment was recorded.

Experiments : Two experiments were conducted with female mice. One group of mice received an amphetamine dose of 2.2 mg/kg and the other 4.4 mg/kg. These doses were based on a previous study showing conditioned place preference in adult male mice and were adjusted for females (Becker et al., 1982; Budygin et al., 2004).

Statistics

Locomotor Activity: Total distance scores of dcc heterozygous and wild-type mice from single dose amphetamine experiments were compared using two-way repeated measure ANOVAs with genotype as the between-group variable and time as the within-group variable. Interaction between mouse genotype and gender was analyzed using a three-way repeated measure ANOVA with genotype and gender as between-group variables and time as the repeated measure variable. Total distance scores for the multiple dose cocaine experiments were compared using a three-way repeated measure ANOVA with genotype and dose as between-group variables and time as the within-group variable. Stereotypy counts were compared using two-tailed Student's t-tests.

Conditioned Place Preference: Place preference was determined by subtracting the time spent in the unpaired compartment from the time spent in the paired compartment. Place preference scores were analyzed using paired Student's t-tests with the significance level adjusted for multiple comparisons using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979).

Results

DCC heterozygous mice show reduced locomotor activity in response to psychostimulants

We previously reported that adult male dcc heterozygous mice of a 129Sv/BL6 mixed genetic background show blunted locomotor responses to a single injection of amphetamine at 1.5, 2.5, or 4 mg/kg (Flores et al., 2005). To determine if this effect is strain-dependent, dcc heterozygous (n=8) and wild-type (n=11) adult male C57BL6 mice were administered a single injection of amphetamine at 2.5 mg/kg. There was no effect of genotype on locomotion or stereotypy following a single injection of saline (Figure 1a,c). Following the injection of amphetamine, dcc heterozygous mice showed significantly reduced locomotor activity compared to wild-type mice ($F_{1,17}=7.71$, $p=0.013$) (Figure 1b). Student's t-tests revealed that dcc heterozygous mice also showed significantly reduced stereotypy counts ($t_{17}=2.40$, $p=0.028$) (Figure 1c).

To assess for gender differences in the locomotor response of dcc heterozygous mice to amphetamine, we examined locomotor activity in adult cycling female dcc heterozygous (n=10) and wild-type (n=10) mice in response to amphetamine. There was no effect of genotype on locomotion or stereotypy following a single injection of saline (Figure 1a,c). We found that female dcc heterozygous mice showed blunted locomotor activity and reduced stereotypy counts in response to a single injection of amphetamine when compared to female wild-type mice (locomotion: $F_{1,17}=12.22$, $p=0.028$; stereotypy: $t_{17}=4.24$, $p=0.0005$) (Figure 1b,c). These results are consistent with male mice, and though a three-way repeated measures ANOVA revealed a significant effect of gender on locomotion after amphetamine treatment ($F_{1,34}=22.46$, $p<0.0001$), there was no interaction between the effects of genotype and gender ($F_{1,34}=0.3$, $p=0.87$).

Finally, we examined locomotor activity in adult cycling female dcc heterozygous and wild-type mice in response to a high (20 mg/kg) or low (10 mg/kg) dose of cocaine. As expected, there was no difference between the dcc heterozygous and wild-type mice following a single injection of saline (Figure 2a,c). Following a single injection of cocaine the locomotor response of the dcc heterozygous mice (10 mg/kg, n=11; 20 mg/kg, n=9) were blunted in comparison to the wild-type mice (10 mg/kg, n=10; 20 mg/kg, n=9) (Figure 2b). A three-way repeated measures ANOVA revealed significant effects of genotype ($F_{1,35}=3.96$, $p=0.05$) and dose ($F_{1,35}=36.30$, $p<0.0001$), indicating a dose-dependent effect. At either dose, dcc heterozygous mice did not significantly

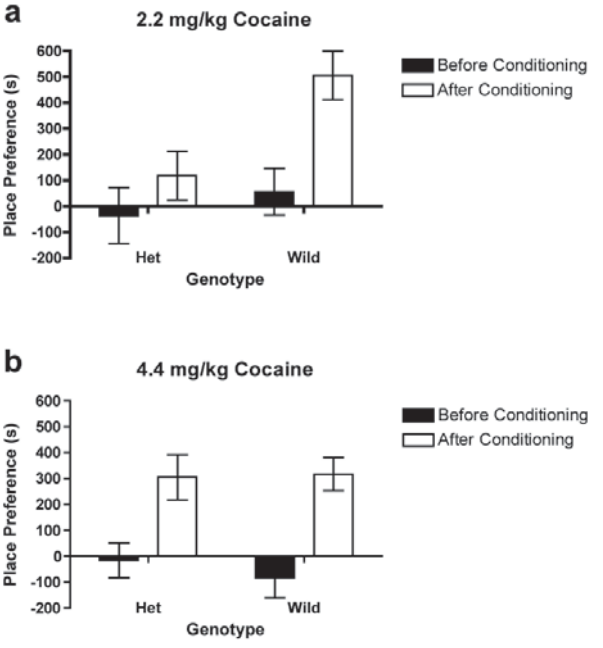

Figure 3: Conditioned place preference for an amphetamine-paired compartment in dcc heterozygous and wild-type mice. Bars represent mean place preference (±standard error means). (a) A low dose of amphetamine induced place preference in wild-type (Wild), but not dcc heterozygous (Het), mice. (b) A high dose of amphetamine induced place preference in both groups.

differ from wild-type mice in stereotypy counts (10 mg/kg, t19=2.00, p=0.06; 20 mg/kg, t16=1.57 p=0.13) (Figure 2d).

In all experiments dcc heterozygous mice showed a blunted locomotor response to the psychostimulant drug administered when compared to their wild-type littermates. They also showed stereotypy counts that were either equivalent to or less than wild-type mice, indicating that the blunted effect on locomotion was not observed due to increased drug-induced stereotypy.

DCC heterozygous mice show reduced sensitivity to the rewarding properties of amphetamine

We determined if amphetamine could induce reward in dcc heterozygous mice. During three days of conditioning adult female dcc heterozygous and wild-type mice (n=10/group) learned to associate one of two distinctive environments with the effects of amphetamine (paired compartment), and the other with saline (unpaired compartment). Preference for the paired compartment during a post-conditioning test, conducted when the mice are in a drug-free state, can be considered as an indication of sensitivity to the rewarding effects of the drug (Tzschentke, 1998). Place preference is expressed as the difference between the times spent in the paired compartment and the unpaired compartment. A positive place preference indicates drug seeking, while a negative place preference indicates drug aversion. Place preference before and after conditioning was compared using paired Student's t-tests with significance level adjusted using the Holm-Bonferroni sequentially rejective procedure (Holm, 1979). At a low dose of 2.2 mg/kg amphetamine, which has previously been shown to induce reward in mice (Budygin et al., 2004), the wild-type mice spent significantly more time in the paired compartment than the unpaired compartment during the post conditioning test (t9=2.88, p=0.018, αadj=0.025). However, dcc heterozygous mice did not show a preference for either compartment (t9=1.3118, p=0.22, αadj=0.05) (Figure 3a). At a higher dose of 4.4 mg/kg both dcc heterozygous and wild-type mice spent significantly more time in the paired compartment (wild-type, t9=3.81, p=0.0042, αadj=0.0125; dcc heterozygotes, t9=3.33, p=0.0088, αadj=0.0167) (Figure 3b).

Discussion

In this study we examined the behavioural phenotype of adult mice that develop with altered netrin-1 signaling. Netrin-1 is a secreted developmental protein involved in directing growing axons towards their synaptic targets (Barallobre et al., 2005). Here, we show that heterozygosity for the netrin-1 receptor dcc protects against the locomotor-activating and rewarding effects of amphetamine and the locomotor-activating effect of cocaine. Given the role of netrin-1 in axon guidance, we believe that altered organization of DA circuitry due to disrupted netrin-1 signaling underlies the observed resistance to these effects of psychostimulants.

A behavioural phenotype in dcc heterozygous mice was first established in mice of 129Sv/BL6 mixed genetic background, which show a blunted locomotor response to amphetamine in a dose-dependent fashion (Flores et al., 2005). Although the current study is an extension of that previous work, here we use C57BL6 strain mice. Since amphetamine-induced behaviours can vary between genetic strains, it is important to determine if the effect of dcc heterozygosity on amphetamine-induced locomotion differs between these two genetic backgrounds. For example, Chen et al. (2007) showed that C57BL6 mice exhibit greater locomotor activation than mice of a 129Sv substrain upon injection of amphetamine. They also showed lower amphetamine-induced striatal DA efflux in 129Sv compared to C57BL6 mice. Here, we demonstrate that dcc heterozygous C57BL6 mice show blunted locomotor activation similar to dcc heterozygous 129Sv/BL6 mice in response to a single injection of amphetamine. Therefore, the blunted amphetamine-induced locomotor activation seen in dcc heterozygous mice is conserved across these genetic strains. It is important to note that while a dose dependent response was observed in 129Sv/BL6 mice, we did not test for dose dependency here. We did, however, find a dose-dependent response to cocaine in C57BL6 mice, which strongly suggests conservation of this component of the dcc heterozygote phenotype as well.

Gender-specific differences in drug-induced locomotor activity have also been observed. For example, Rojas et al. (2007) reported a stronger locomotor response to phencyclidine (PCP) in wild-type C57BL6 female mice compared to male mice. Although they did not observe a gender difference in response to amphetamine, other studies have observed an effect of gender on amphetamine-induced locomotion (Suiciak et al., 2007). This raised the possibility that the behavioural phenotype of dcc heterozygous mice in response to amphetamine may manifest differently in males and females. To address this, we investigated drug-induced locomotor activation in cycling adult female dcc heterozygous and wild-type mice in response to amphetamine. We found that the altered amphetamine-induced locomotor response seen in adult male mice is also seen in adult female mice, indicating conservation of the phenotype across genders in the adult. Despite significantly lower locomotor activation in female mice as compared to males, there was no interaction between sex and genotype. As this behavioural result is consistent between male and female dcc heterozygous mice, female mice were used for further behavioural testing.

The relationship between increased locomotion and DA release in the NAcc is well documented. Locomotor activation in response to amphetamine can be blocked by lesioning the NAcc and by local injection of haloperidol, a neuroleptic DA antagonist (Teitelbaum et al., 1979). In the absence of amphetamine, direct DA injection into the NAcc is sufficient to enhance locomotion (Jenkins and Jackson, 1986). Recent microdialysis data collected in our laboratory showed that blunted amphetamine-induced locomotor activation seen in dcc heterozygous mice is correlated with reduced DA release in the NAcc (Grant et al., 2007). This finding supports the idea that locomotor activation can be used as an indication of the "state of function" of the mesolimbic DA system.

Increased DA signaling in the NAcc is a common psychostimulant effect; however, psychostimulants employ multiple molecular mechanisms to achieve this result. To examine whether dcc heterozygous mice show a blunted locomotor response to a psychostimulant that acts by a different mechanism of action than amphetamine, we investigated their response to cocaine. We found that cocaine reduces locomotor activation in dcc heterozygous mice in a dose-dependent fashion, paralleling the response to amphetamine (Flores et al., 2005). These results suggest that NAcc DA signaling is reduced in dcc heterozygous mice in response to cocaine as well as amphetamine. This provides evidence that the behavioural phenotype of dcc heterozygous mice is conserved across psychostimulants that act via amphetamine-like and cocaine-like mechanisms. Amphetamine-like drugs act mainly by inducing the release of DA from storage vesicles

into the synaptic cleft. Cocaine-like mechanisms prevent the reuptake of DA, thereby increasing the amount of DA in the synaptic cleft (Riddle et al., 2005; Geracitano et al., 2006). It would be interesting to assess the locomotor response of dcc heterozygous mice to additional drugs in both categories as well as to test the cocaine response of dcc heterozygous mice to other aspects of behaviour that are altered in response to cocaine in wild-type mice, such as protection against sensorimotor gating deficits (Doherty et al., 2007). These avenues of study are currently being investigated in our laboratory.

In addition to locomotor activation, psychostimulants have been shown to induce reward in humans as well as mice (Budygin et al., 2004, Tilley et al., 2007). The rewarding properties of a drug can be tested using a conditioned place preference paradigm (Tzschenkte, 1998). In this paradigm, drug administration is repeatedly paired with a compartment that has distinctive visual and tactile features. A second compartment with different visual and features is paired with a control substance, i.e. saline. When allowed to move between the drug-paired compartment and the saline-paired compartment, a mouse that showed no pre-conditioning preference for either compartment will spend more time in the drug-paired compartment after drug treatment. This drug-seeking behaviour is present only when the drug has a rewarding effect for the mouse (Tzschenkte, 1998; Sanchis-Segura & Spanagel, 2006). In dcc heterozygous mice we found no drug seeking behaviour at a dose of amphetamine that elicited drug seeking behaviour in their wild-type littermates. Interestingly, at a higher dose of amphetamine drug-seeking behaviour was observed in both wild-type and dcc heterozygous mice. This raises the possibility that dcc deficiency produces a rightward shift in sensitivity to the rewarding properties of amphetamine in the adult mouse.

Psychostimulant-induced reward, like locomotor activation, is mediated by increased extracellular DA in the NAcc (Sellings and Clarke, 2003; Di Chiara et al., 2004). Interestingly, although DA activity in the NAcc is reduced in dcc heterozygous mice following an amphetamine injection, DA activity and tyrosine hydroxylase (TH) expression in the medial prefrontal cortex (mPFC) are heightened in response to amphetamine, as compared to wild-type mice (Flores et al., 2005; Grant et al., 2007). Given that DA activity in the prefrontal cortex has an inhibitory effect on DA release in the NAcc, it is likely that DA release in the NAcc is reduced as a result of DA hyperactivity in the mPFC (Herve et al., 1981; Deutch et al., 1990; Doherty and Gratton, 1996; Beyer and Steketee, 1999; Ventura et al., 2004, Flores et al., 2005; Grant et al., 2007). This suggests that higher levels of extracellular DA in the mPFC can account for the reduced behavioural responses to cocaine and amphetamine seen in adult dcc heterozygous mice. Dopaminergic projections to the mPFC and NAcc share a common origin, the ventral tegmental area. Indeed, the two pathways, mesolimbic and mesocortical, are commonly referred to together as the mesocorticolimbic DA system (Le Moan & Simon, 1991). As midbrain DA neurons express DCC, and based on our current and previous behavioural and biochemical findings, we hypothesize that the mPFC DA hyperactivity in dcc heterozygous mice is the result of abnormal mesocorticolimbic DA neuronal development, which, in turn, is due to disrupted netrin-1 signaling (Flores et al., 2005; Osborne et al., 2005; Grant et al., 2007).

Due to an incomplete understanding of the biochemical, cellular, and behavioural consequences of DCC deficiency, we cannot be certain as to how DCC deficiency affects the functioning of the mesocorticolimbic DA system. Modulation to aspects of DA function in addition to DA release, such as synthesis, metabolism and signal attenuation, may also contribute to the abnormal DA signaling seen in dcc heterozygous mice. Alterations to brain structures known to innervate the mesocorticolimbic DA system, such as the hippocampus and amygdala, could also contribute to abnormal DA function (Grace et al., 2007). Aberrations in these structures have been observed in developing dcc homozygous mice during embryogenesis, though it is currently unknown if similar defects are present in dcc heterozygotes or if these embryonic aberrations would result in altered structures in the adult (Barallobre et al., 2000). Furthermore, other neurotransmitters known to modulate drug-induced DA activity in NAcc, such as norepinephrine and glutamate, could contribute to abnormal DA function (Ventura et al., 2003; Grace et al., 2007; Rommelfanger and Weinshenker, 2007). However, previous studies appear indicate that the major component of norepinephrine signaling pathway is unaltered in dcc heterozygous mice. Our laboratory has found normal expression of dopamine-beta-hydroxylase, the enzyme that converts DA to norepinephrine, in the mPFC of dcc heterozygous mice, and no difference in amphetamine-induced extracellular norepinephrine in the prefrontal cortex (Grant et al., 2007). Future studies further examining the potential roles of other neurotransmitters and brain structures on DA signaling in dcc heterozygous mice are currently being investigated by our laboratory.

Studies have shown that slight alterations in genetic and environmental factors during brain development may increase or decrease an individual's susceptibility to the effects of psychostimulant drugs as adults (De Wit, 1998; Piazza et al., 1998; Suzuki et al., 2003; Ventura et al., 2004; Miyatake et al., 2006; Reichel et al., 2006; Fujii et al., 2007). Here, we find that altering the expression of DCC leads to a phenotype that appears protected from the rewarding and locomotor-activating effects of the psychostimulant amphetamine. At least one aspect of this phenotype, locomotor activation, is consistently protected across gender, two genetic strains and two mechanisms of drug action, indicating a robust phenotypic expression of the dcc haploid genotype. In addition, these mice appear protected against the rewarding effects of amphetamine. These findings suggest that changes in the expression of the netrin-1 receptors can alter behavioural responses in a very specific manner, which supports our hypothesis that variable expression of netrin-1 receptors can create variations in behaviour between individuals. Due partly to its importance in drug addiction, the development and function of the mesocorticolimbic DA system is an area of intense scrutiny and study in neuroscience. Our findings point to DCC as a critical determinant of mesocorticolimbic DA function and of susceptibility to the effects of psychostimulant drugs.

Acknowledgments

We thank S. Ackerman (The Jackson Laboratory) for the original dcc heterozygous breeders, T. Pawson (University of Toronto) for the pan-UNC-5 antiserum, L. Yetnikoff, R. Kyle and A. Arvanitogiannis for guidance and critical readings of the manuscript, and C. Himmelman and Z. Speed for their excellent technical assistance. This work was funded by the Canadian Institute for Health Research (C.F.), the Natural Science and Engineering Research Council of Canada (D.H., C.F., A.G.), and the Fonds de la Recherche en Santé du Québec (C.F.).

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