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***Dcc* Haploinsufficiency Regulates Dopamine Receptor Expression Across Postnatal Lifespan**

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Abstract

Adolescence is a period during which the medial prefrontal cortex (mPFC) undergoes significant remodeling. The netrin-1 receptor, DCC, controls the extent and organization of mPFC dopamine connectivity during adolescence and in turn directs mPFC functional and structural maturation. *Dcc* haploinsufficiency leads to increased mPFC dopamine input, which causes improved cognitive processing and resilience to behavioral effects of stimulant drugs of abuse. Here we examine the effects of *Dcc* haploinsufficiency on the dynamic expression of dopamine receptors in forebrain targets of C57BL6 mice. We conducted quantitative receptor autoradiography experiments with [³H]SCH-23390 or [³H]raclopride to characterize D1 and D2 receptor expression in mPFC and striatal regions in male *Dcc* haploinsufficient and wild-type mice. We generated autoradiograms at early adolescence (PND21±1), mid-adolescence (PND35±2), and adulthood (PND75±15). C57BL6 mice exhibit overexpression and pruning of D1, but not D2, receptors in striatal regions, and a lack of dopamine receptor pruning in the mPFC. We observed age- and region-specific differences in D1 and D2 receptor density between *Dcc* haploinsufficient and wild-type mice. Notably, neither group shows the typical pattern of mPFC dopamine receptor pruning in adolescence, but adult haploinsufficient mice show increased D2 receptor density in the mPFC. These results show that DCC receptors contribute to the dynamic refinement of D1 and D2 receptor expression in striatal regions across adolescence. The age-dependent expression of dopamine receptor in C57BL6 mice shows marked differences from previous characterizations in rats.

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Author Contributions

M.P. analyzed and interpreted data and wrote the manuscript. A.G. performed experiments and wrote the manuscript. F.S. acquired data. Y.D. provided training and expertise. C.M. contributed to the conception of the project and data analysis. C.F. conceived and designed the study, supervised the project, and wrote the manuscript.

Keywords

netrin-1; DCC; guidance cues; prefrontal cortex; adolescence; receptor pruning

Adolescence is a critical period of development marked by drastic changes in gross brain morphology and fine-scale connectivity. In humans, structural imaging studies show significant alterations in cortical thickness and connectivity at this age (Gogtay et al., 2004, Sowell et al., 2004). In laboratory animals, studies reveal region-specific differences in synaptic pruning and neurotransmitter receptor expression (Rakic et al., 1986, Andersen et al., 2000). Dopamine connectivity in the mPFC, specifically, undergoes substantial reorganization and functional refinement during the adolescent period (Kalsbeek et al., 1988, Benes et al., 2000, Tarazi and Baldessarini, 2000, Manitt et al., 2011, Naneix et al., 2012). Growing evidence suggests that the protracted development of dopamine circuitry in the prefrontal cortex controls the maturation of behavioral inhibition and decision-making displayed by adults across species, as well as the increased risk for psychopathologies associated with impairments in these cognitive processes (Spear, 2000, Sturman and Moghaddam, 2011, Naneix et al., 2012). Perturbations of mesocortical dopamine development in rodents result in alterations in adult mPFC function and behaviors similar to symptoms of psychiatric disorders such as increased impulsivity, exaggerated salience attribution, and impaired cognitive function (Goldstein and Volkow, 2011, Bortolato et al., 2015).

Our group has identified the netrin-1 receptor, Deleted in Colorectal Cancer (*Dcc*), as the first gene involved specifically in the adolescent maturation of mPFC dopamine connectivity. During adolescence, DCC receptor signaling within dopamine neurons controls the extent of dopamine innervation to the mPFC (Manitt et al., 2013). DCC receptors in turn: (a) dictate the structure and excitability of adult mPFC pyramidal neurons (Grant et al., 2007, Manitt et al., 2011), (b) fine-tune cognitive flexibility and behavioral inhibition (Manitt et al., 2013, Reynolds et al., 2015b) and (c) determine the control that mPFC dopamine exerts over mesolimbic dopamine neurons (Pokinko et al., 2015). *Dcc* haploinsufficiency leads to increased mPFC dopamine input, which causes improved cognitive processing and resilience to the behavioral effects of stimulant drugs of abuse in adulthood (Grant et al., 2007, Reynolds et al., 2015a). These traits only emerge after adolescence and are opposite to those observed in psychopathologies such as schizophrenia, drug addiction, and major depressive disorder (Grant et al., 2009, Manitt et al., 2013, Yetnikoff et al., 2014, Volkow and Morales, 2015, Flores et al., 2016). Notably, *DCC* haploinsufficiency has recently been identified in human populations (Srouf et al., 2010, Meneret et al., 2014).

To date we have demonstrated a role for DCC receptors in the development of the dopamine projections to the mPFC in adolescence. However, whether and how variations in *Dcc* expression affect the dynamic regulation of dopamine receptors across postnatal lifespan is unknown. This is particularly important because striatal dopamine receptor function during adolescence is known to influence mesocortical dopamine function in adulthood (Kellendonk et al., 2006). Here, we examined for the first time the density of D1 and D2

receptors in forebrain regions from adolescence until adulthood (from here on referred to as “postnatal lifespan”) in C57BL6 mice. We find overexpression and pruning of D1, but not D2, receptors in striatal regions, and no dopamine receptor pruning in the mPFC. We then show that DCC receptors play a role in the dynamic pattern of dopamine receptor expression using our mouse model of *Dcc* haploinsufficiency.

EXPERIMENTAL PROCEDURES

Animals

Male *Dcc* haploinsufficient mice originally obtained from Dr. S. Ackerman (The Jackson Laboratory) were maintained on the C57BL6 background in our animal colony. For all experiments, we compared male *Dcc* haploinsufficient to wild-type littermate controls. Mice were kept on a 12 h light/dark cycle with ad libitum access to food and water. Animals were weaned at postnatal day (PND) 20 and housed with same-sex littermates. All experiments were performed in accordance with the guidelines set forth by the Canadian Council of Animal Care and by the Animal Care Committees of McGill University/Douglas Mental Health University Institute.

Quantitative receptor autoradiography

Tissue processing—The expression of dopamine receptors in mesocorticolimbic dopamine terminal regions was assessed using quantitative receptor autoradiography as described previously (Hersi et al., 1995). Briefly, brains from male *Dcc* haploinsufficient mice and wild-type littermates were collected at PND21±1, PND35±2, and PND75±15. We define these ages as early adolescence, mid-adolescence, and adulthood. We want to note, however, that these are not absolute margins, but age ranges during which mice exhibit distinct neurobehavioral characteristics (Spear, 2000; Schneider, 2013; Manitt et al., 2013, Reynolds et al., 2015a).

Brains were sectioned using a cryostat (16 µm thick slices) and thaw-mounted on Superfrost Plus slides (Thermo Fisher, Waltham, MA, USA). Sections were collected in a 2:6 manner (for every six sections, we collected two contiguous sections on separate slides; one slide was used to measure total binding, the second to assess non-specific binding). Slides were stored at −80°C until radiolabeled.

Autoradiography—D1 and D2 receptors were radiolabeled as previously described (Hersi et al., 1995). Briefly, slides were incubated with ligands diluted in Tris buffer at room temperature for 60 minutes. For D1 receptor, total radioligand binding was determined using [³H]-SCH 23390 (1 nM; Perkin Elmer, Waltham, MA, USA). To measure the degree of non-specific binding, the contiguous sections were incubated in the same buffer, but supplemented with 1 µM unlabeled SCH23390 (Sigma-Aldrich, St. Louis, MO, USA). D2 receptor binding was determined using [³H]-raclopride (2.85 nM; Perkin Elmer). Non-specific binding of the D2 radioligand was assessed in adjacent sections that were incubated in the same buffer supplemented with 1 µM (+)-butaclamol (Sigma-Aldrich). Test cassettes containing slices from each age and genotype, each with its own [³H] standard, were used to

ensure proper exposure time and signal strength. Representative autoradiograms are shown in Fig. 1.

Densitometric analysis—Radiolabeled slides and calibrated [^3H] standards were exposed to Hyperfilm (Kodak, Rochester, NY, USA) for either 10 weeks (D1 receptor) or 13 weeks (D2 receptor). Optical density measurements were made on a computerized image analyzer (MCID System, Imaging Research Inc., St. Catharines, ON, CA). Three sections were selected from the pregenual forebrain of each mouse, corresponding to Plates 17, 19 and 21 from the mouse brain atlas (Paxinos and Franklin, 2008). Regions of interest were traced in both hemispheres and consisted of the pregenual mPFC, dorsal striatum, and nucleus accumbens (NAcc). The sampling area of the mPFC included the cingulate, prelimbic, and infralimbic subregions. The sampling area for the NAcc included the core and shell. [^3H] standards were used to generate a standard curve, and the density of radioligand bound per milligram of tissue was computed based on specific radioactivity (fmol/mg $\pm$ SEM). Specific binding was determined by subtracting non-specific binding from total binding. Data from both hemispheres were pooled since no inter-hemispheric differences were detected.

Statistical analysis—Differences in receptor density for each anatomical region were analyzed using two-way Analysis of Variance (ANOVA) with genotype and age as between-group variables. Bonferroni post-hoc comparisons were performed on significant effects. At specific ages, further comparisons of cortical and striatal subregions were performed using planned individual t-tests (two tailed). Non-significant p values are expressed as not significant (n.s). Group sizes are indicated in the figure captions.

RESULTS

Dopamine receptor expression in the NAcc

Dopamine D1 receptors—D1 receptor levels peak at PND35 in the NAcc of both wild-type and *Dcc* haploinsufficient mice, with no significant differences between genotypes (Fig. 2A; two-way ANOVA, main effect of age: $F_{(2,32)} = 8.17$, $p < 0.002$, no effect of genotype, no significant genotype $\times$ age interaction. Post hoc analysis for effect of age showed increased D1 levels at PND35 compared to both PND21 and adulthood, $p < 0.05$). Because of functional and anatomical differences between the lateral and medial portions of the NAcc, we thought that perhaps differences between genotypes may be masked at PND35. Thus, we chose to analyze dopamine receptor expression in wild-type and *Dcc* haploinsufficient mice in the medial and lateral NAcc at PND35. Remarkably, we find that D1 receptor density is reduced at PND35 in the medial, but not lateral, NAcc of *Dcc* haploinsufficient mice compared to controls. (Fig. 2A inset; Student's t-test, medial NAcc: $t_{(13)} = 4.16$, $p < 0.002$; lateral NAcc: n.s).

Dopamine D2 Receptors—D2 receptor levels in the NAcc remain unchanged across postnatal lifespan in C57BL6 mice. D2 receptor expression differs between *Dcc* haploinsufficient mice and wild-type controls, but only at PND21 (Fig. 2B; two-way ANOVA, no effect of age, no effect of genotype, significant age $\times$ genotype interaction

$F_{(2,34)} = 3.39$, $p < 0.05$. Post hoc analysis revealed a significant difference between *Dcc* haploinsufficient and wild-type mice at PND21, $p < 0.05$). Further analysis of D2 expression in medial and lateral NAcc subregions at PND21 shows a significant reduction of D2 receptor levels in both subregions (Fig. 2B inset; Student's t-test, medial NAcc: $t_{(11)} = 2.37$, $p < 0.05$; lateral NAcc: $t_{(11)} = 2.32$, $p < 0.05$).

Dopamine receptor expression in the dorsal striatum

Dopamine D1 receptors—D1 receptor levels in the dorsal striatum of wild-type mice peak at PND35 before decreasing in adulthood, in line with previous reports in rats (Teicher et al., 1995, Naneix et al., 2012). However, this overproduction is not observed in *Dcc* haploinsufficient mice (Fig. 3A; two-way ANOVA, main effect of age: $F_{(2,32)} = 3.50$, $p < 0.05$, main effect of genotype: $F_{(1,32)} = 7.52$, $p < 0.01$, no significant genotype $\times$ age interaction. Post hoc analysis for age showed increased D1 levels between PND21 and PND35, $p < 0.05$, and between PND35 and adulthood, $p < 0.01$). Comparisons of dorsal striatal subregions show differences in D1 levels between *Dcc* haploinsufficient and wild-type mice at PND35 in both the medial and lateral dorsal striatum (Figure 3A inset; Student's t-tests, medial dorsal striatum: $t_{(13)} = 3.30$, $p < 0.01$; lateral dorsal striatum: $t_{(13)} = 2.85$, $p < 0.02$).

Dopamine D2 Receptors—The density of D2 receptors in the dorsal striatum increases steadily across postnatal lifespan in both wild-type and *Dcc* haploinsufficient mice, with no indication of the adolescent pruning present in rats (Teicher et al., 1995, Tarazi and Baldessarini, 2000) (Fig. 3B; two-way ANOVA, main effect of age: $F_{(2,32)} = 3.91$, $p < 0.05$, no effect of genotype, no significant genotype $\times$ age interaction. Post hoc analysis for effect of age revealed a significant difference between D2 levels at PND21 and adulthood, $p < 0.05$). Comparison between medial and lateral dorsal striatal subregions reveal that *Dcc* haploinsufficiency reduces D2 receptor expression in the medial dorsal striatum at PND21 (Fig. 3B inset; Student's t-tests, medial dorsal striatum: $t_{(11)} = 3.40$, $p < 0.01$; lateral dorsal striatum: n.s.).

Dopamine receptor expression in the mPFC

Dopamine D1 receptors—We find D1 receptor levels in the mPFC remain the same across all ages in both wild-type and *Dcc* haploinsufficient C57BL6 mice, in contrast to previous reports in rats (Andersen et al., 2000). No differences were detected in D1 expression in the mPFC between *Dcc* haploinsufficient mice and wildtype controls at any age (Fig. 4A; two way ANOVA, no effect of age; no effect of genotype; no significant genotype $\times$ age interaction). However, we have previously shown that variations in *Dcc* expression exert preferential effects on dorsal mPFC circuitry (Manitt et al., 2011, Manitt et al., 2013). Furthermore, an effect at PND21 may be masked by high variability within the *Dcc* haploinsufficient group. We therefore compared receptor density specifically within dorsal and ventral mPFC subregions at PND21. We observed a reduction in D1 receptor density at PND21 between *Dcc* haploinsufficient and control mice in the dorsal, but not ventral, mPFC (Fig. 4A inset; Student's t-test, dorsal mPFC: $t_{(8)} = 2.76$, $p < 0.05$; ventral mPFC: n.s.).

Dopamine D2 Receptors—D2 receptor expression in the mPFC varies across life span in both *Dcc* haploinsufficient and wild-type mice. (Fig. 4B; two-way ANOVA, main effect of age: $F_{(2,26)} = 5.99$, $p < 0.01$, no effect of genotype, no significant genotype $\times$ age interaction. Post hoc analysis for effect of age showed a significant difference in D2 receptor density between PND35 and adulthood, $p < 0.01$). Because *Dcc* expression has subregion-specific effects on mPFC circuitry in adult mice (Manitt et al., 2011, Manitt et al., 2013), we examined dorsal and ventral subregions of the adult mPFC separately for effects of genotype. Subregion-specific analysis of D2 expression in adulthood shows increased D2 in the ventral, but not dorsal, mPFC of in *Dcc* haploinsufficient mice compared to wild-type (Fig. 4B inset; Student's t-tests, dorsal mPFC: n.s; ventral mPFC: $t_{(12)} = 2.20$, $p < 0.05$).

DISCUSSION

In this study we describe, for the first time, the expression pattern of D1 and D2 receptors in forebrain targets of dopamine neurons in C57BL6 mice across postnatal development. Moreover, we show that the dynamic pattern of receptor expression is altered in *Dcc* haploinsufficient mice in an age- and region-specific manner. In wild-type mice, D1 receptor levels in the NAcc and dorsal striatum increase from PND21 to PND35 but decrease in adulthood, similar to rats (Tarazi and Baldessarini, 2000). In contrast, D2 receptor expression increases steadily with age in the dorsal striatum, but not NAcc. Notably, we did not observe overproduction or pruning of dopamine D1 or D2 receptors in the mPFC, contrary to previous reports in rats (Andersen et al., 2000). Remarkably, *Dcc* haploinsufficiency alters the density of D1 and D2 receptors in an age- and region-dependent manner. At PND21, *Dcc* haploinsufficiency leads to reduced D2 receptor levels in the NAcc and the medial dorsal striatum. At PND35, it attenuates the overproduction of dopamine D1 receptors in the medial NAcc and the dorsal striatum. In the cortex, *Dcc* haploinsufficient mice exhibit transiently decreased density of D1 receptors in the dorsal mPFC at PND21, but increased D2 receptor density in the ventral mPFC in adulthood. To our knowledge, this is both the first description of dopamine receptor expression in C57BL6 mice and the first identification of a gene involved in directing the refinement of dopamine receptors in limbic forebrain regions during adolescence.

D1 and D2 receptor levels in the NAcc and dorsal striatum

Dopamine D1 receptor levels peak at PND35 throughout the dorsal striatum and NAcc of C57BL6 mice before decreasing in adulthood. In rats, a similar pattern of overexpression and pruning of D1 receptors during mid-adolescence is present in the dorsal striatum, but is markedly diminished in the NAcc (Teicher et al., 1995, Tarazi and Baldessarini, 2000). *Dcc* haploinsufficient mice do not show D1 receptor overproduction at PND35 in the dorsal striatum or medial NAcc, in line with their phenotype of reduced sensitivity to psychostimulant drugs of abuse (Grant et al., 2007), which emerges in adulthood (Grant et al., 2009, Yetnikoff et al., 2011, Yetnikoff et al., 2014). Because DCC receptors in striatal areas are expressed exclusively by dopamine axons (Manitt et al., 2011), we believe changes in striatal receptor density in *Dcc* haploinsufficient mice are due to dopamine fibers growing through these areas during adolescence (Kalsbeek et al., 1988). We have previously proposed that reduced DCC leads to target recognition errors by mesolimbic dopamine

axons and their netrin-1 expressing postsynaptic targets in adolescence (Grant et al., 2007, Manitt et al., 2013). This may in turn modify normal D1 and D2 receptor expression in the striatum during development, as striatal dopamine receptor availability depends on proper dopamine signaling in early life (Kostrzewa and Saleh, 1989).

D2 receptor levels in C57BL6 mice increase steadily across the **postnatal** lifespan in the dorsal striatum, but remain unchanged in the NAcc. Previous findings in rats on this topic are somewhat inconsistent: striatal D2 receptors may increase until adulthood or peak in mid-adolescence before being pruned, an effect which varies across the mediolateral axis and is attenuated in the NAcc (Teicher et al., 1995, Tarazi and Baldessarini, 2000). Intriguingly, *Dcc* haploinsufficiency leads to a transient reduction of D2 receptors throughout the NAcc and in the medial dorsal striatum at PND21. This is particularly significant as recent studies suggest alterations in striatal D2 receptor levels in adolescence may be critical for the development of mPFC circuitry. In mice, increased striatal D2 receptor levels during adolescence impair dopamine neuron activity (Krabbe et al., 2015), causing deficits in working memory and behavioral flexibility in adulthood (Kellendonk et al., 2006). This suggests a mechanism by which reduced DCC, and in turn reduced striatal D2 receptor density in early adolescence, may contribute to the enhanced cognitive processing and blunted striatal dopamine release observed in *Dcc* haploinsufficient mice (Grant et al., 2007, Manitt et al., 2013, Reynolds et al., 2015b). Studies are currently ongoing to determine whether DCC contributes to the expression of striatal dopamine receptors in humans, and how this may affect cognitive function.

D1 and D2 receptor levels in the mPFC

We do not observe overproduction or pruning of D1 or D2 receptors in the mPFC of C57BL6 mice in adolescence, unlike previous reports in rats (Andersen et al., 2000, Naneix et al., 2012). However, assessment at additional adult ages would be required to conclude that no reduction occurs later in adulthood. Future studies will more thoroughly assess dopamine receptor levels at ages PND100 and above. Nonetheless, there is a lack of consensus on this topic as a number of rat and human studies find no dopamine receptor pruning in cortical areas (Tarazi Baldessarini, 2000, Rothmond et al., 2012). Since D1 receptor expression may depend on endogenous dopamine signaling early in life (Kostrzewa and Saleh, 1989), the low concentrations of extracellular dopamine in the mPFC characteristic of the C57BL6 mouse strain (Ventura et al., 2004) may attenuate dopamine receptor expression in the mPFC across **postnatal** lifespan. It is nonetheless of interest to note that DCC has age-dependent effects on D1 and D2 receptor levels throughout life.

The fact that *Dcc* haploinsufficiency results in a transient reduction in D1 receptor density in the dorsal mPFC at PND21 is especially intriguing because at this age dopamine axons have not fully innervated the mPFC. In rodents and humans, DCC receptors are expressed throughout life by mPFC pyramidal and GABA cells in addition to dopamine neurons (Torres-Berrio et al., 2016, Manitt et al., 2010, Manitt et al., 2011). Recent studies implicate DCC expression within hippocampal pyramidal neurons in the regulation of N-methyl-D-aspartate (NMDA) receptor function (Horn et al., 2013), which may in turn affect D1 receptor trafficking, availability, and stability (Cepeda and Levine, 2006). This suggests

DCC may alter D1 receptor expression intrinsically in mPFC GABA or pyramidal neurons prior to the maturation of mesocortical dopamine circuitry. It is not clear why this effect is more pronounced in dorsal, rather than ventral, mPFC, though data suggest DCC reorganizes the mPFC with a dorsal-to-ventral bias (Manitt et al., 2011, Manitt et al., 2013).

We believe the delayed increase in D2 receptor density in the ventral mPFC of adult *Dcc* haploinsufficient mice is likely postsynaptic, as mesocortical dopamine projections sparsely express D2 mRNA (Lammel et al., 2008). In both rodents and primates, D2 receptor activation in adulthood inhibits cortical pyramidal cell activity, enhances cortical signal-to-noise ratio, and promotes behavioral flexibility and associative learning (Seamans and Yang, 2004, O'Donnell, 2010, Arnsten et al., 2015). Increased D2 expression is therefore consistent with observations that adult *Dcc* haploinsufficient mice show reduced excitability of mPFC pyramidal neurons and increased performance in cognitive tasks (Manitt et al., 2013). In humans, behavioral disinhibition and impaired cognitive function are typical of schizophrenia, drug abuse, and major depressive disorder (Austin et al., 2001, Goldstein and Volkow, 2011, Lesh et al., 2011). The role of D2 receptors in these psychopathologies is conflicting: while early publications showed disorganized pattern of expression and reduced cortical D2 receptors in pathologies such as schizophrenia (Goldsmith et al., 1997, Suhara et al., 2002), recent imaging studies find no differences in baseline mPFC D2 receptor binding in schizophrenia or major depressive disorder (Saijo et al., 2010, Slifstein et al., 2015). Nonetheless, evidence indicates an association between genetic variation in *DCC* and schizophrenia (Grant et al., 2012, Yan et al., 2016), and increased *DCC* mRNA in the mPFC of patients with major depressive disorder who died by suicide (Manitt et al., 2013, Torres-Berrio et al., 2016). Together, our data suggest that DCC may affect vulnerability or resilience by altering cortical D2 receptor expression and that *Dcc* haploinsufficiency may be a suitable model to investigate the role of cortical D2 receptors in psychopathology.

Our previous studies show that female mice with *Dcc* haploinsufficiency show similar phenotypes than those observed in male mice (Grant et al., 2007; Grant et al., 2009). Thus, the changes in D1 and D2 receptor expression we report in this study may also occur in females. In the future we plan to test this possibility directly.

CONCLUSION

Here, we characterize dopamine receptor expression in forebrain target regions of dopamine neurons across postnatal lifespan of C57BL6 mice. We show that DCC receptors exert age- and region-specific effects on dopamine receptor levels throughout postnatal development: *Dcc* haploinsufficiency transiently reduces dopamine receptor levels in striatal and cortical regions during early adolescence, but leads to a lasting upregulation of D2 receptors in the adult mPFC. These results may have important implications regarding our understanding of the mechanisms underlying dopamine receptor regulation and pruning in adolescence, and the process by which risk factors in adolescence may disrupt the function of corticolimbic circuitries. Our findings further highlight variations in *Dcc* expression as an important mediator of susceptibility or resilience to dopamine-related psychopathologies via alterations in dopamine receptors and circuitry.

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Abbreviations

ANOVA	analysis of variance
DCC	deleted in colorectal cancer
mPFC	medial prefrontal cortex
NAcc	nucleus accumbens
PND	postnatal day

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HIGHLIGHTS

- DCC receptors control dopamine D1 and D2 receptor expression in prefrontal cortex and striatal regions across the lifespan
- *Dcc* haploinsufficiency leads to enduring increases in D2 receptor expression in the prefrontal cortex
- C57BL6 mice do not exhibit the dopamine receptor pruning in the prefrontal cortex previously described in rats

Fig. 1.
Representative autoradiograms. Plates correspond to Plates 17, 19 and 21 from the mouse brain atlas (Paxinos and Franklin, 2008). Regions of interest were traced in both hemispheres and consisted of the pregenual medial prefrontal cortex, dorsal striatum, and nucleus accumbens. Autoradiograms shown were obtained from adult brain sections incubated with [³H]-SCH 23390.

Nucleus Accumbens

D1 receptor

D2 receptor

Fig. 2.

Dopamine receptor expression in the nucleus accumbens across lifespan. (A) Total D1 receptor density (D1; fmol/mg protein) in nucleus accumbens (NAcc) showed a main effect of age, and did not differ between *Dcc* haploinsufficient (*Dcc* haplo) and wild-type mice. Post hoc analysis for age shows increased D1 levels at PND35 compared to both PND21 and adulthood (PND21: *Dcc* haplo n=6, wild-type n=4; PND35: *Dcc* haplo n=6, wild-type n=10; Adult: *Dcc* haplo n=7, wild-type n=5). **Inset:** Further analysis of medial and lateral NAcc shows a significant decrease in D1 levels in medial, but not lateral, NAcc subregions of *Dcc*

haplo compared to wild-type mice at PND35 (medial NAcc: *Dcc* haplo n=5, wild-type n=10; lateral NAcc: *Dcc* haplo n=6, wild-type n=9). **(B)** Total D2 receptor density (D2; fmol/mg protein) in NAcc shows no effect of age or genotype, but a significant age $\times$ genotype interaction. Post hoc analysis reveals significantly reduced D2 density in *Dcc* haplo compared to wild-type mice at PND21. (PND21: *Dcc* haplo n=7, wild-type n=4; PND35: *Dcc* haplo n=3, wild-type n=7; Adult: *Dcc* haplo n=6, wild-type n=5). **Inset:** Further analysis of medial and lateral NAcc at PND21 shows a significant decrease in D2 levels in both the medial and lateral NAcc of *Dcc* haplo compared to wild-type mice (medial NAcc: *Dcc* haplo n=6, wild-type n=5; lateral NAcc: *Dcc* haplo n=7, wild-type n=7). *p < 0.05, **p < 0.01. All data are presented as mean $\pm$ SEM.

Fig. 3.

Dopamine receptor expression in dorsal striatum across lifespan. **(A)** Total D1 receptor density (D1; fmol/mg protein) in dorsal striatum shows a main effect of age and a main effect of genotype. Post hoc analysis shows increased D1 levels at PND35 compared to both PND21 and adulthood (PND21: *Dcc* haplo $n=6$, wild-type $n=4$; PND35: *Dcc* haplo $n=5$, wild-type $n=10$; adult: *Dcc* haplo $n=7$, wild-type $n=6$). **Inset:** Further analysis of medial and lateral dorsal striatal subregions shows reduced D1 levels in both the medial and lateral dorsal striatum of *Dcc* haplo compared to wild-type mice at PND35 (medial dorsal striatum:

Dcc haplo n=5, wild-type n=10; lateral dorsal striatum: *Dcc* haplo n=5, wild-type n=10). **(B)** Total D2 receptor density (D2; fmol/mg protein) in dorsal striatum shows a main effect of age. Post hoc analysis shows an increase in D2 receptor density between PND21 and adulthood (PND21: *Dcc* haplo n=8, wild-type n=6; PND35: *Dcc* haplo n=3, wild-type n=8; Adult: *Dcc* haplo n=8, wild-type n=5). **Inset:** Further analysis of medial and lateral dorsal striatal subregions shows decreased D2 levels in medial dorsal striatum of *Dcc* haplo compared to wild-type mice at PND21 (medial dorsal striatum: *Dcc* haplo n=8, wild-type n=5; lateral dorsal striatum: *Dcc* haplo n=8, wild-type n=5). * $p < 0.05$, ** $p < 0.01$. All data are presented as mean $\pm$ SEM.

Fig. 4.

Dopamine receptor expression in pregenual medial prefrontal cortex across lifespan. **(A)** Total medial prefrontal cortex (mPFC) D1 receptor density (D1; fmol/mg protein) shows no effect of age and does not differ between *Dcc* haploinsufficient (*Dcc* haplo) and wild-type mice (PND21: *Dcc* haplo n=6, wild-type n=4; PND35: *Dcc* haplo n=6, wild-type n=9; Adult: *Dcc* haplo n=7, wild-type n=6). **Inset:** Separate analysis of dorsal and ventral mPFC subregions reveals reduced D1 levels in dorsal, but not ventral, mPFC of *Dcc* haplo compared to wild-type mice at PND21 (Dorsal mPFC: *Dcc* haplo n=6, wildtype n=4; Ventral

mPFC: *Dcc* haplo n=6, wild-type n=4). **(B)** Total mPFC D2 receptor density (D2; fmol/mg protein) shows a main effect of age, but no genotype or genotype $\times$ age interaction. Post hoc analysis shows increase in D2 receptor levels between PND35 and adulthood (PND21: *Dcc* haplo n=7, wild-type n=4; PND35: *Dcc* haplo n=3, wild-type n=7; Adult: *Dcc* haplo n=6, wild-type n=5). **Inset:** Further analysis of dorsal and ventral mPFC in adulthood reveals increased D2 levels in the ventral, but not dorsal, mPFC of adult *Dcc* haplo mice compared to wild-type (Dorsal mPFC: *Dcc* haplo n=6, wild-type n=5; Ventral mPFC: *Dcc* haplo n=7, wild-type n=7). * $p < 0.05$. All data are presented as mean $\pm$ SEM.