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Making Dopamine Connections in Adolescence

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Abstract

A dramatic maturational process ongoing in adolescence is prefrontal cortex development, including its dopamine innervation. Dopamine axons grow from the striatum to the prefrontal cortex, the only known case of long-distance axon growth during adolescence. This is coordinated by the Netrin-1 guidance cue receptor DCC, which in turn controls the intrinsic development of the prefrontal cortex itself. Stimulant drugs in adolescence alter DCC in dopamine neurons and in turn prefrontal cortex maturation, impacting cognitive abilities. Variations in DCC expression are linked to psychiatric conditions of prefrontal cortex dysfunction, and microRNA regulation of DCC may be key to determining adolescent vulnerability or resilience. Since early interventions are proving to effectively ameliorate disease outcome, the Netrin-1 system is a promising therapeutic target.

Keywords

prefrontal cortex; Netrin; DCC; guidance cues; stimulant drugs; microRNA

Adolescence as a period of behavioural and cognitive transition

Adolescence is the time when individuals must negotiate the transition between childhood and adulthood at several levels (molecular, cellular, physical, social). This transition requires a diverse knowledge and skill set pertaining to independent living and the ability to navigate emotional, social and psychological stimuli far more varied and complex than those experienced during childhood. As such, adolescence is characterized by dramatic behavioural and cognitive changes.

Concurrent with and underlying these adolescent changes is ongoing brain development, in particular at the level of the **prefrontal cortex** (see Glossary). This region, heavily involved in the complex cognitive and social processes that mature during adolescence, continues to develop into early adulthood [e.g. 1]. One of the key changes to the prefrontal cortex during adolescence is that its dopamine input continues to increase [2–6]. This dopamine growth in the prefrontal cortex is unique; maximum innervation levels of other neuromodulators are

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typically reached prior to the onset of adolescence [7]. Furthermore, protracted dopamine innervation to the prefrontal cortex is critically important for various aspects of prefrontal function, including working memory, affective processing, reasoning, planning, attention, inhibitory control and the calibration of risk and reward [e.g. 8]. Not surprisingly, these are among the cognitive processes that are still maturing throughout adolescence [9]. In fact, adolescent changes in some cognitive functions seem to occur in parallel to mesocortical dopamine maturation [3,10,11].

As prefrontal dopamine fibres continue to increase in density during adolescence, they may be particularly vulnerable to external influences, both positive and negative. Alterations in dopamine connectivity and activity have been linked to cognitive dysfunction observed across various psychiatric conditions [12]. The delayed dopamine connectivity in the prefrontal cortex during adolescence may underlie, at least in part, the heightened risk to develop mental disorders [13]. Therefore external influences that can affect adolescent prefrontal dopamine development may have the potential either to exacerbate or protect against the risk of developing certain psychiatric conditions.

In order to understand both the cognitive changes that occur during adolescence as well as adolescent vulnerability to mental disorders, it is crucial to clarify the cellular and molecular mechanisms underlying dopamine innervation and connectivity in the prefrontal cortex. Recent studies using rodent model systems have pointed at the role of specific axon **guidance cues** in this context. This review examines these advances, which lead to a molecular mechanistic explanation for the protracted development of mesocortical dopamine projections. We propose that this emerging picture could explain, at least in part, the particular susceptibility of adolescents to drug addiction and other mental disorders.

The challenges of characterizing adolescence across species

Though we may think of adolescence as a characteristically human attribute, the key behavioural traits associated with the adolescent transition are remarkably consistent across diverse species. Adolescence in humans is characterised by specific, well-described behavioural changes, particularly increased sociality, increased novelty-seeking, and increased risk-taking [14], and these changes are seen in a range of other animal species. The advantage of this is that these organisms can be used to study the neural changes associated with adolescence. However, a challenge to overcome is the need to precisely characterize the developmental time period that constitutes adolescence across multiple species, while keeping in mind that adolescence is a gradual behavioural and cognitive shift that does not have a specific onset or offset.

In the scientific literature there is a specific developmental timeframe typically referred to as the “adolescent period”. This is more of an arbitrary standard that encloses adolescence rather than the ‘true’ adolescent period. In the case of mice and rats, the adolescent period is usually considered to encompass the time from when the animals are weaned until they reach their adult weight [14–19]. In humans most often it is considered to be between 12 and 19 years of age [14,20]. These adolescent periods are often further divided into three equal thirds constituting “early,” “mid,” and “late” adolescence. Standardisation of the adolescent

period, with all its caveats, allows for reproducible and quantifiable comparisons across species, particularly between model organisms, in our case mice, and humans, whose development we ultimately strive to understand.

Dopamine development in the prefrontal cortex is complex and critical

In the prefrontal cortex all cortical layers are innervated by dopamine fibres, which synapse onto pyramidal cells and parvalbumin-expressing interneurons [21–25]. However, the density of fibres varies between layers: density is greater in the superficial and deep cortical layers compared to the intermediate layers [25]. In the rodent, superficial layer dopamine fibres are distinct from deep layer fibres; they are located only in the supragenual (posterior) parts of the cingulate 1 and cingulate 2 subregions and originate from the substantia nigra rather than the ventral tegmental area [2,26]. To our knowledge, studies on the function of this separate yet interesting population of dopamine fibres are lacking. Therefore, we will limit our discussion to the much better known fibres located in the deep layers in the rodent. These fibres are located in the pregenual (anterior) medial prefrontal cortex and the orbital prefrontal cortex, and originate from cell bodies located in the ventral tegmental area.

Dopamine neurons in the ventral tegmental area, in addition to projecting to cortical regions, also project to subcortical structures, including the nucleus accumbens. This **mesocorticolimbic dopamine system** governs key aspects of complex behaviour including motivation and reward. However, despite their common origin, mesocortical and mesolimbic projections are distinct: they originate from different ventral tegmental area neuronal populations with specific structures and physiologies [27]. Furthermore, dopamine input to the prefrontal cortex is sparse compared to the dense input to limbic structures like the nucleus accumbens [3,28]. Nonetheless, we and others have shown that mesocortical dopamine fibres appear to be strategically localized to profoundly influence prefrontal cortex function [29–31].

Dopamine connectivity is still in flux during adolescence

The vast majority of neuronal growth, maturation, and connectivity happens during the prenatal and juvenile life stages. By the onset of adolescence, most axonal innervations in the brain have reach their adult densities, including dopamine innervations to limbic regions [32,33]. Although this is true of the nucleus accumbens, there is ample evidence that adolescence is a dynamic period in terms of connectivity and receptor expression in this region in particular [34–36]. In contrast, the dopamine innervation to the prefrontal cortex continues to increase throughout adolescence, achieving final axon density levels in early adulthood [2,3,6,28]. This is accompanied by increases in dopamine synapses onto prefrontal pyramidal neurons [5].

The unknown source of the increasing mesocortical dopamine fibres

It has long been known that the dopamine fibre density in the prefrontal cortex continues to increase throughout adolescence, however the source of this increase was unknown. The most parsimonious explanation was that dopamine axons innervating the cortex continue to

sprout new branches throughout adolescence. However, an alternate possibility was that additional dopamine axons continue to reach the cortex throughout this period. Such late-stage, long-distance growth would make the dopamine innervation to the prefrontal cortex all the more unusual.

To study this possibility, our group developed a strategy using dual viral injections to label and track axon growth [31]. Using this strategy, we labelled dopamine axons that innervate the nucleus accumbens at the onset of adolescence. When we tracked these axons into adulthood we found that, though the vast majority of axons remained in the nucleus accumbens, a significant subset grew to the medial prefrontal cortex [31]. We also found that, as more dopamine axons innervate the medial prefrontal cortex, there is a correlated reduction in the axons innervating the nucleus accumbens [31]. This was the first clear evidence that protracted mesocortical dopamine development is due to ongoing axonal growth. Because they grow such a long distance, mesocortical dopamine axons need to make frequent and cumulative navigational decisions at intermediate targets and likely remain particularly vulnerable to environmental influences throughout this process.

Netrin-1 and its receptors coordinate neural development throughout life

What are the molecular mechanisms guiding adolescent mesocortical axon development, and are they linked to the intrinsic maturation of the prefrontal cortex? During neural development, growing axons find their targets by responding to the coordinated actions of proteins called guidance cues. Guidance cues vary in effective concentration, both spatially across the brain and temporally throughout development, forming signalling pathways that guide growing axons to their intended targets. One family of guidance cues, found across all bilaterally symmetrical animals, is the netrins [37]. Work from our group has focused on the most characterized protein in the netrin family, **Netrin-1**, and its role is mesocortical axon growth and navigation.

Netrin-1 is a ~65 kDa protein with 3 domains [38]. The two N-terminal domains (domains VI and V) are homologues to domains in the laminin protein family, while the C-terminal domain appears unique to netrins and binds to cell surfaces and the extracellular matrix [38,39]. Netrin-1 acts as a guidance cue by influencing the “decision-making” processes of growing axons, effectively signalling whether, where and when to grow [39–42]. Netrin-1 also plays a critical role in the wiring events that follow axonal pathfinding, including target recognition, axon arborisation, and synapse formation, as well as in other processes like oligo-axonal adhesion and synaptic plasticity [38,43–45]. The different functions achieved by Netrin-1 appear to be specific to particular developmental periods and maturational states, including adulthood [44,46].

Netrin-1 organizes neuronal circuitries by either attracting or repelling growing axons and dendrites [37]. These opposing responses depend on the binding of Netrin-1 to different receptors or receptor complexes. Two families of Netrin-1 receptors, the **DCC** (deleted in colorectal cancer) and the **UNC5** (uncoordinated 5) families, account for the bifunctional nature of Netrin-1. DCC receptors mediate attraction, whereas UNC5 receptors signal repulsion [47]. These opposing actions can be altered by regulating the availability of DCC

and UNC5 receptors at the cell surface [48]. Therefore, the selectivity with which Netrin-1 organizes neuronal connectivity can be mediated by the balance between “attractive” and “repulsive” receptors expressed at a given time. Subtle alterations in receptor expression can result in selective changes to the organization of neuronal circuits and, in turn, significant changes to the function of these systems in adulthood. As discussed next, our group has shown that variations in DCC receptor expression during adolescence dramatically influence the development of the rodent mesocorticolimbic dopamine system.

Mapping the topography of Netrin-1 and DCC in mesocorticolimbic dopamine targets

Dopamine neurons in the ventral tegmental area highly express DCC receptors throughout life [49,50]. During embryonic rodent development, the onset of DCC receptor expression appears to coincide with dopamine axons starting to grow towards the forebrain [50–52]. DCC continues to be strongly expressed by ventral tegmental neurons throughout postnatal development and to a lesser degree in adulthood [50].

In the medial prefrontal cortex, DCC receptors are expressed by pyramidal neurons [53], but only weakly by dopamine axons [28]. Here, Netrin-1 expression is substantial and localized to pyramidal and parvalbumin-expressing GABA neurons [28], the neurons onto which dopamine axons synapse. Notably, Netrin-1 expression is particularly strong in the deep cortical layers [28], where dopamine innervation is dense. In contrast, in the nucleus accumbens DCC receptors are exclusively expressed by dopamine axons and Netrin-1 expression is much weaker [28]. These contrasting expression patterns are maintained before, during and after adolescence [28]. The high-to-low, receptor-to-ligand expression of DCC and Netrin-1 in dopamine targets suggests an important role for these proteins in the development, organization, and connectivity of mesocorticolimbic dopamine neurons (Figure 2). We hypothesize that axons expressing copious DCC are sensitive to low concentrations of Netrin-1, while axons expressing minute quantities of DCC respond only to higher concentrations of Netrin-1.

To uncover the actual relationship between Netrin-1 signalling and adolescent dopamine development, we have studied the consequences of varying *Dcc* gene expression with spatial and temporal specificity. We have used *Dcc* heterozygous mice as well as mice with ***Dcc* haploinsufficiency** exclusively in dopamine neurons from adolescence onwards [28,29,31,46]. As DCC regulates many processes throughout life, we used *Dcc* heterozygous mice to gain an initial understanding of the role of DCC in adolescence and to develop more specific, testable hypotheses. To confirm the role of the mesocorticolimbic system, all critical results we describe have been generated or replicated using mice with targeted *Dcc* haploinsufficiency in dopamine neurons from adolescence onwards. This strategy has uncovered a critical role for DCC signalling in shaping mesocorticolimbic dopamine circuitry. Notably, *Dcc* haploinsufficiency also occurs in humans [54–58].

DCC receptors control dopamine axon targeting in adolescence

As mentioned, DCC is highly expressed by mesolimbic dopamine axons [28]. By inducing *Dcc* haploinsufficiency specifically in dopamine neurons during adolescence and tracking the growth of their axons, the role of DCC in determining the trajectory of these axons can be studied [31]. Dopamine axons with reduced *Dcc* fail to recognize the nucleus accumbens as their intended innervation target and instead reroute to the inner layers of the medial prefrontal cortex [31]. This results in excess dopamine varicosities and dopamine content in the prefrontal cortex [28,29,31,49,59]. Because prefrontal dopamine varicosities are the sites at which the majority of dopamine synthesis, packaging, release and reuptake occur [60], and because in the cortex about 93% of dopamine varicosities form synapses [61], it is possible that DCC receptor signalling ultimately controls the number of dopamine synapses in the medial prefrontal cortex. In summary, DCC receptor signalling promotes target recognition of mesolimbic dopamine axons in the nucleus accumbens, thereby controlling the extent of dopamine innervation to the medial prefrontal cortex (Figure 3, Key Figure).

DCC-mediated wiring of mesocortical dopamine circuitry determines complex cognition in adulthood

By delimiting mesocortical dopamine input, DCC receptors determine the dendritic arbour complexity, dendritic spine density, and excitability of prefrontal layer V pyramidal neurons [28,29,31,49]. Because of these developmental alterations, we hypothesized that DCC signalling in adolescence influences adult cognitive abilities associated with prefrontal cortex function, specifically **cognitive inhibition** and behavioural flexibility. To examine cognitive inhibition, we tested mice heterozygous for *Dcc* in dopamine neurons in a Go/No-Go task, in which the animals are trained to respond to a specific cue, as well as to refrain their behavioural responses according to the presence of a secondary cue in order to obtain a food reward [62]. These mice show equal performance at all training stages of the task, but during the Go/No-Go phase show a specific behavioural alteration; while they have a normal ability to respond during a go trial, they are better able to inhibit responses during a no-go trial [31], indicating that these animals are less impulsive. We also found improved cognition in *Dcc* heterozygous mice in the attentional **set-shifting** test [63], a test of cognitive flexibility. Cognitive or behavioural flexibility is the ability to make adaptive responses to a changing environment and is specifically tested by the extradimensional set-shifting component of the attentional set-shifting test [29,63,64]. Performance on this component of the test is enhanced in *Dcc* heterozygous mice [29]. In contrast, these mice perform normally in the other stages of the task, including intradimensional set-shifting, indicating that their ability to generate an **attentional set** is not altered [29,63]. By reducing DCC expression on dopamine neurons, we have been able to manipulate the adolescent development of the prefrontal cortex and selectively alter prefrontal cortex-dependent behaviours.

By organizing dopamine development, DCC receptors determine adult sensitivity to stimulant drugs

Dopamine neurotransmission in the prefrontal cortex plays an important role in regulating dopamine activity in the nucleus accumbens. In fact, prefrontal dopamine activity has been shown to attenuate accumbal dopamine release [65]. Because we found that DCC controls dopamine connectivity and activity in the prefrontal cortex, we hypothesized that it may also impact the effects of stimulant drugs that are mediated by dopamine release in the nucleus accumbens. These include effects on locomotor activity, conditioned place preference, sensorimotor gating, and sensitivity to intracranial self-stimulation. We have found that, as adults, mice with *Dcc* haploinsufficiency are protected against all these effects [49,59,66–68]. Furthermore, we recently demonstrated that increased input and concentration of dopamine in the medial prefrontal cortex directly causes the effects on locomotor activity [69], and we expect that the same is true for the other effects.

By inducing *Dcc* haploinsufficiency in dopamine neurons in adolescence, dopamine axons are rerouted to the prefrontal cortex. As noted earlier, this results in a measureable decrease in the dopamine innervation to the nucleus accumbens [31]. This reduction may also contribute to the blunted amphetamine-induced dopamine release in the nucleus accumbens and therefore the blunted behavioural responses.

Addictive drugs in adolescence alter DCC signalling and dopamine development

Adolescent exposure to factors that increase the risk of developing disorders involving prefrontal cortex dysfunction, such as addictive drugs, may cause their effects by altering DCC expression in dopamine neurons. We have shown that repeated exposure to amphetamine in adolescence, but not in adulthood, downregulates DCC receptors in ventral tegmental area dopamine neurons [70,71]. Remarkably, adult mice that received this treatment during adolescence have an increase in the dopamine input to the prefrontal cortex [72]. Importantly, the drug regimen we use results in blood plasma levels in mice comparable to those achieved by adolescent people who use amphetamine recreationally [73]. As we detailed above, reduced DCC expression in adolescence induces target-recognition errors by dopamine axons in the nucleus accumbens and their ectopic innervation in the prefrontal cortex [31]. Therefore, we believe that the increased span in mesocortical innervation observed in amphetamine-treated mice may result from drug-induced target recognition errors in the nucleus accumbens and concomitant rerouting to the prefrontal cortex (Figure 3c).

It is important to emphasize that although amphetamine in adolescence leads to an increase in the expanse of dopamine axons in the medial prefrontal cortex, it also induces a dramatic reduction in the number and density of their presynaptic sites [72]. This effect is opposite to the one observed in transgenic mouse models of *Dcc* haploinsufficiency [28,29]. How amphetamine denudes dopamine axons of presynaptic sites remains to be established. However, aberrant contact and adhesion between dopamine axon terminals and dendrites in

the prefrontal cortex is likely to be involved [74]. This idea is consistent with evidence showing that DCC-mediated Netrin-1 signalling maintains synapses in the prefrontal cortex [75].

Variations in DCC receptor expression are linked to psychiatric disorders

Dcc haploinsufficiency has been found in several family lineages worldwide [54–58]. There has been great interest in the neuroanatomical and behavioural implications of *Dcc* haploinsufficiency in humans, however its implications for mesocorticolimbic development and related psychiatric disorders have so far remained unexamined. We have started to address these questions, and we predict that *Dcc* haploinsufficiency will alter mesocorticolimbic connectivity. We expect that this change will be associated with differences in prefrontal cortex-dependent cognitive functions such as cognitive flexibility, reasoning, planning, and reward calibration. We anticipate phenotypic changes to emerge after adolescence. Furthermore it would be interesting to determine whether subjects with *Dcc* haploinsufficiency show some level of protection against disorders of prefrontal cortex dysfunction.

Recently, using techniques such as genome-wide association, several studies have suggested links between DCC receptors and psychiatric conditions. One intriguing finding was an association between schizophrenia and polymorphisms in the 3′ **untranslated region** (UTR) of the *DCC* gene [76,77]. Although associations between gene polymorphisms and schizophrenia are not uncommon, this finding may be of particular significance because post-transcriptional gene expression regulators modify gene expression by binding the 3′ UTR [78], and therefore such polymorphisms could alter DCC expression levels in dopamine neurons and disrupt mesocorticolimbic system development. A second notable link between psychiatric disease and altered DCC epigenetics is an increase of about 50% in DCC gene expression in the prefrontal cortex of antidepressant-free patients with major depressive disorder who committed suicide [29], a finding later replicated [53]. In fact, there is now substantial correlational genetic evidence from human populations that variation in *DCC* expression is a risk factor for depression [29,53,79–82]. Although such evidence lacks a causative link, using a mouse model of depression we have recently shown that increased *Dcc* expression in the prefrontal cortex induces susceptibility to developing depression-like phenotypes [53]. This is the first direct evidence that DCC plays a causal role in determining vulnerability to traits that are associated with psychiatric conditions.

The increase in *DCC*-expression found in subjects with depression is negatively correlated with the expression of microRNA-218, a potent repressor of DCC expression in human and mouse neurons [53]. **MicroRNAs** are emerging as molecular links between environmental factors and changes in gene expression [78] and are commonly altered in psychiatric disorders [83]. In general, microRNAs can be relatively easily measured in blood samples [84], so it is feasible that microRNA-218 could be measure in humans. Since circulating levels of microRNAs, including microRNA-218, are known to correlate with levels of microRNAs in the brain, blood-sample measurements of microRNAs, including perhaps microRNA-218, could potentially serve as biomarkers for susceptibility to psychiatric disorders [84,85]. This is a promising strategy for identifying people, particularly at the

onset of adolescence, who may be predisposed to developing psychiatric disorders, including depression. Providing interventions for these individuals *prior* to any prodromal symptoms could offer an effective new way to combat adolescent-emergent psychiatric disorders.

Concluding Remarks

Mesocortical dopamine axons represent the only known case of long-distance axon growth during adolescence. As these axons navigate to the prefrontal cortex they remain particularly sensitive to environmental influences. The Netrin-1/DCC signalling pathway, which mediates dopamine axon targeting decisions, is a molecular substrate linking environmental events in adolescence to changes in brain maturation and cognition. Notably, animal studies have shown that stimulant drugs acting via Netrin-1/DCC can modify the extent and the organization of the dopamine input to the prefrontal cortex. Furthermore, changes in DCC expression, including in humans, most likely involving microRNAs, are implicated in psychiatric disorders. As microRNAs can be easily measured in blood samples, this presents an opportunity to identify individuals, at a vulnerable age, who may be at risk of developing a psychiatric condition. Contrasting with the detrimental effects of changes in DCC expression, work using transgenic *Dcc* mouse models has also shown the potential of alterations in DCC expression to be protective. Identifying potential environmental influences that can induce these beneficial alterations is an active area of research (see Outstanding Questions). Perhaps the most intriguing of the avenues for further investigation is the possibility of preventing the development of psychiatric conditions in humans through targeted *a priori* interventions. Much further research would be needed to achieve this goal, both to pin down useful biomarkers and to identify effective interventions. Nonetheless, the key contributions of the Netrin-1/DCC pathway to prefrontal cortex development during adolescence, and accordingly its relevance to psychiatric disorders, are becoming clear.

Outstanding Questions

- To what extent does adolescent dopamine axon growth contribute to individual differences in vulnerability to psychiatric disorders? Do variations in *DCC* gene expression dictate susceptibility to psychiatric disorders? What is the role of microRNAs?
- Does DCC/Netrin-1 signalling play a role in the changes to the endocrine system that coincide with adolescence? If so, is there any interaction between the roles of Netrin/DCC in dopamine and endocrine development?
- Do fluctuations in circulating gonadal hormone levels influence the effects of DCC receptors on dopamine axon targeting in adolescence? Are these effects different between males and females?
- Does exposure to other drugs, such as alcohol and marijuana, during adolescence influence the growth of dopamine axons? And what about exposure to amphetamine at doses used in therapeutic contexts; do they influence the growth of dopamine axons?

- Can resilience to psychopathology be induced through changes in the Netrin-1/DCC signalling pathway? If so, what environmental experiences could trigger these processes?
- Is there a role for the other known Netrin-1 receptors, UNC-5 (uncoordinated), Neogenin, and DSCAM (Down syndrome cell adhesion molecule), in coordinating dopamine axon growth in adolescence? UNC-5C receptors are expressed in dopamine neurons from adolescence onwards. What are the implications of this for axon targeting?
- By controlling the extent of the dopamine input to the prefrontal cortex, DCC receptors determine the functional organization of pyramidal neurons. Does the Netrin-1/DCC system influence the adolescent maturation of GABA interneurons as well?
- Why does late-stage axon growth exist in the brain? Does it have an evolutionary benefit?

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Glossary

Attentional Set
a group of related stimuli that either do or do not contain information relevant to a task, such as acquiring food. By classifying individual stimuli into sets, information processing and decision making can be performed more efficiently.

Cognitive Inhibition
the ability to ignore stimuli that do not contain information relevant to a task, even if the stimuli are relevant to a different task. The **Go/No-Go Task** is a commonly used test of this ability.

DCC
the acronym for the protein “deleted in colorectal cancer”; acts as a Netrin-1 receptor. It is expressed by growing axons and signals attraction to Netrin-1.

***Dcc* haploinsufficient mouse models**
genetically modified mice that carry one non-functional allele of the *Dcc* gene. These mice have reduced DCC protein expression in the brain compared to wild-type mice. More refined manipulations can be used to modulate DCC expression in specific populations of neurons and/or time windows. For instance, the Cre/lox viral-mediated gene editing technique has been used to generate mice with *Dcc* haploinsufficiency only in ventral tegmental dopamine neurons and from adolescence onwards.

Guidance Cue
a protein which signals the appropriate path for growing neurites to follow to their intended innervation target through the developing nervous system.

Mesocorticolimbic Dopamine System
a group of brain regions consisting of the ventral tegmental area and forebrain regions that receive its dopamine projections. The "**mesolimbic**" component of this system refers to the limbic forebrain regions that receive these projections and includes the nucleus accumbens. The "**mesocortical**" component of this system refers to the cortical forebrain regions that receive these projections and consists primarily of the prefrontal cortex.

microRNA
a small RNA that prevents the translation of a specific messenger RNA into protein by binding the 3′ untranslated region. MicroRNAs are thought to be important for the "fine tuning" of protein expression.

Netrin-1
A guidance cue protein that binds to elements of the extracellular matrix and interacts with receptors expressed on the surfaces of growing axons, signalling axon growth trajectories. Netrin-1 is also heavily involved in other aspects of axon development and maintenance and is expressed in the brain throughout life.

Prefrontal Cortex
The most anterior component of the frontal lobe of the cerebral cortex; defined anatomically by the dense projection it receives from the dorsomedial thalamic nucleus. Heavily involved in the most complex components of cognition including planning, reasoning and decision making. Receives a sparse dopamine input from the ventral tegmental area that continues to increase until early adulthood.

Set-shifting
the process of learning that the information contained by different attentional sets has changed. The "dimension" of an attentional set refers to the sensory modality or property of the set, for example colour, texture, shape, or odour. Set-shifting may be intra- or extradimensional.

UNC5
the acronym for the protein "uncoordinated-5"; acts as a Netrin-1 receptor. When bound to Netrin-1 it forms a complex with DCC and signals repulsion from regions of high Netrin-1. Whether the mammalian forms of UNC5 can respond to Netrin-1 in the absence of DCC remains unknown.

Untranslated region (UTR)
messenger RNA nucleotide sequences that do not consist of codons and are not translated, but instead contain regulatory sequences. They can be located before the start codon (5′) or after the stop codon (3′).

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Trends

- Dopamine axons grow from the nucleus accumbens to the prefrontal cortex during adolescence; this is the only known case of adolescent long-distance axon growth
- The Netrin-1 receptor DCC is responsible for coordinating this growth
- These axons are particularly vulnerable to environmental effects, including recreational drug use, during adolescence and are strategically localized to profoundly influence prefrontal cortex structure and function
- Changes in *DCC* gene expression in humans are linked psychiatric conditions involving prefrontal cortex dysfunction, including schizophrenia and depression
- *DCC* gene expression is under microRNA control, which may act as a link between environmental factors and prefrontal cortex development

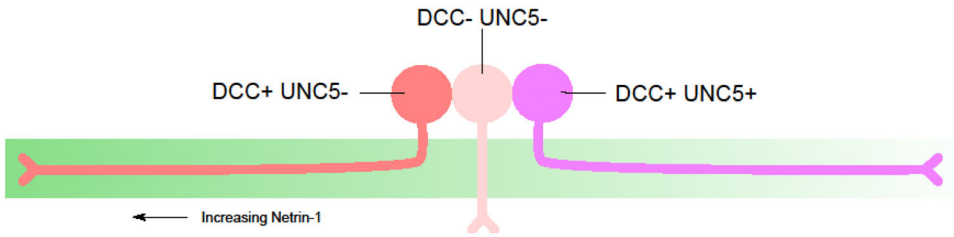

Figure 1. The responses of growing axons to Netrin-1 gradients

The response of growing axons to Netrin-1 depends on the expression pattern of Netrin-1 receptors on the axons. In general, axons that express DCC but not UNC5 (DCC+ UNC5−) are attracted to Netrin-1, whereas axons that express both DCC and UNC5 (DCC+ UNC5+) are repelled by Netrin-1. Axons that express neither DCC nor UNC5 (DCC− UNC5−) do not respond to the Netrin-1 gradient, provided they do not express other Netrin-1 receptors.

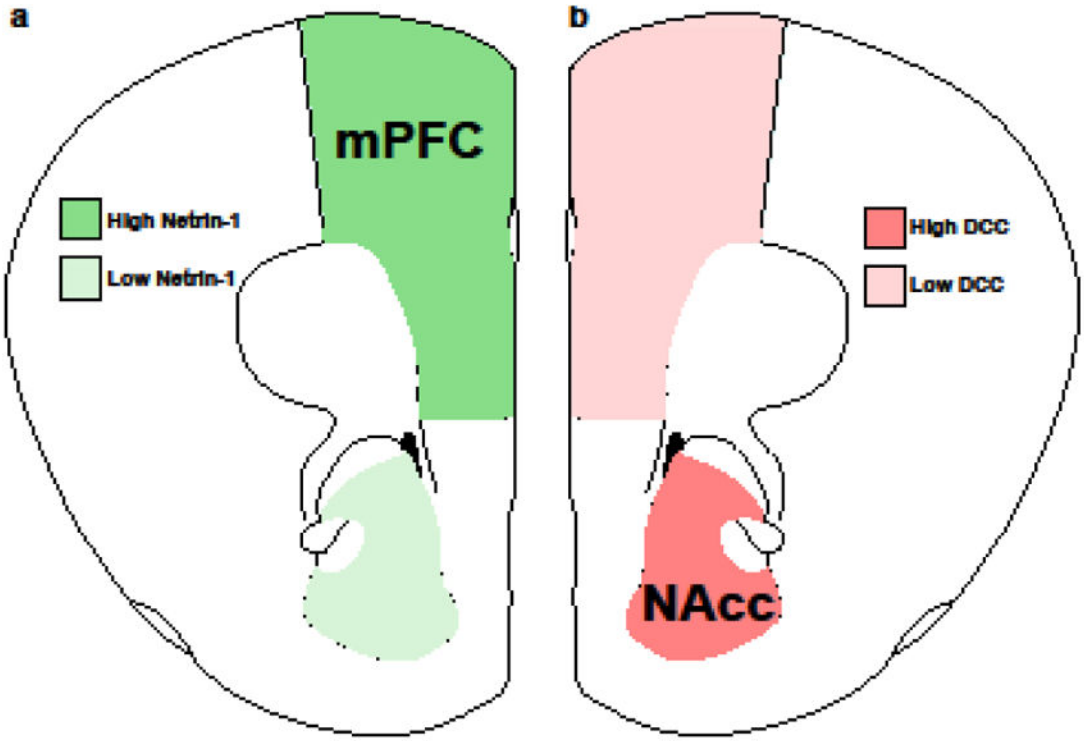

Figure 2. Schematic illustration of the complementary expression patterns of Netrin-1 and DCC in forebrain dopamine innervation targets

The figure depicts a coronal section through the anterior mouse brain (modified from Paxinos & Franklin [86]). The figure illustrates only the relative levels of DCC expressed by dopamine axons, not DCC expressed by other cell types. **(a)** Netrin-1 expression is substantial in the medial prefrontal cortex (mPFC) and much weaker in the nucleus accumbens (NAcc). **(b)** Dopamine axons in the mPFC rarely express DCC. In contrast, DCC is highly expressed in the nucleus accumbens. Note that in this figure the frontal 2 cortical region, also referred to as secondary motor cortex, is included in the mPFC. Despite its name, this region is considered part of the prefrontal cortex [87].

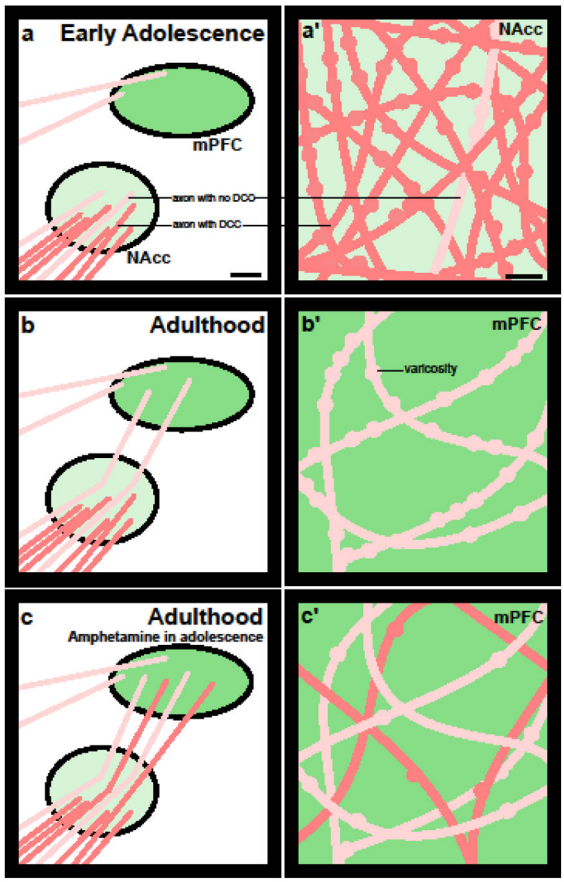

Figure 3, Key Figure. DCC expression in dopamine axons determines their forebrain innervation target in adulthood

(**a**) A schematic diagram of the nucleus accumbens (NAcc, light cyan) and the medial prefrontal cortex (mPFC, dark green) shows that at the onset of adolescence, both dopamine axons that express DCC (red) and ones that do not (pink) innervate the nucleus accumbens. Dopamine axons that innervate the mPFC by early adolescence do not pass through the NAcc. scale bar = approximately 500μm (**a**′) A zoomed-in illustration of the nucleus accumbens at the onset of adolescence showing the high density of dopamine input to this region. This level of dopamine innervation remains relatively unchanged throughout adolescence and adulthood. scale bar = approximately 10μm (**b**) In the adult, dopamine axons that do not express DCC have left the NAcc and now innervate the mPFC. (**b**′) A zoomed-in illustration of the mPFC emphasizes that the density of dopamine innervation is far lower than in the NAcc. (**c**) A schematic diagram representing ectopic innervation of dopamine fibres in the adult mPFC following exposure to amphetamine in adolescence. (**c**′) A zoomed-in illustration of the adult mPFC following exposure to amphetamine in adolescence demonstrates that although there is an increase in the number of dopamine axons innervating the mPFC, these axons differ from the ones seen in non-treated animals: they are denuded of varicosities, and some of the innervating axons, abnormally, express DCC.