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The Netrin-1/DCC guidance cue pathway as a molecular target in depression: Translational evidence

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Abstract

The Netrin-1/DCC guidance cue pathway plays a critical role in guiding growing axons towards the prefrontal cortex (PFC) during adolescence and in the maturational organization and adult plasticity of PFC connectivity. In this review we put forward the idea that alterations in PFC architecture and function, which are intrinsically linked to the development of major depressive disorder (MDD), originate in part from the dysregulation of the Netrin-1/DCC pathway by a mechanism that involves the microRNA, miR-218. We discuss evidence derived from mouse models of stress and human postmortem brain and genome-wide association studies (GWAS), indicating an association between the Netrin-1/DCC pathway and MDD. We propose a potential role of circulating miR-218 as a biomarker of stress vulnerability and MDD.

Keywords

Prefrontal cortex; microRNA; Neurodevelopment; Resilience; Adolescence; Biomarkers

I. INTRODUCTION

Major depressive disorder (MDD) is a debilitating and potentially fatal psychiatric condition affecting ~5% of the world population and contributing significantly to the global burden of disease and disability(1). The treatments for MDD remain inadequately effective for roughly half of patients due to our limited understanding of the molecular mechanisms underlying this disorder and the heterogeneity of its symptomalogy(2). which can be experienced as early as in periadolescence and remains present across the lifespan(3–7). Postmortem and

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neuroimaging studies have consistently demonstrated that increased vulnerability to depression is associated with altered organization of the prefrontal cortex (PFC) circuitry, including its reciprocal connections with the ventral tegmental area (VTA), nucleus accumbens (NAcc), hippocampus (HPC), and amygdala, among other regions (8–12). These structural alterations comprise impaired signaling of proteins that are essential for neuronal morphology and synaptic plasticity, resulting in cortical dysfunction (13,15,17).

The fine organization of neuronal circuits is achieved during embryonic and postnatal life through the action of guidance cues, families of proteins that direct growing neurites (e.g., axons or dendrites) toward their intended targets(16,18–20). Guidance cues, including the netrin, ephrin, slit, and semaphorin families, bind to specific receptors located at the tip of the growth cone (the enlarged protrusion of the growing neurite) to promote neurite extension (attraction) or collapse (repulsion) by a mechanism involving dynamic remodeling of the actin cytoskeleton(21).

Netrin-1, the most characterized member of the netrin family and a member of the laminin superfamily, organizes neural network connectivity bifunctionally—promoting attraction or repulsion, depending on the activation of different receptors or receptor complexes(19,22,23). There are two main families of Netrin-1 receptors, DCC (deleted in colorectal cancer) and UNC5 (uncoordinated-5) (Figure 1A). DCC receptors account for attraction, whereas UNC5 receptors alone or as DCC/UNC5 complexes mediate repulsion (Figure 1A)(24–26). Netrin-1 influences axonal navigation by signaling whether, when, and where to grow (27–29) and also plays a critical role in post-axonal pathfinding wiring events, including axon arborization, dendritic growth, and synapse formation(30–36). These functions appear to be specific to particular developmental periods and maturational states (Figure 1B) (37,38) and maybe be sensitive to positive and negative environmental events(38–43).

Variations in Netrin-1 or DCC/UNC5 receptors during critical periods for the establishment of motivation- and reward-relevant circuitries are likely to contribute to individual differences in susceptibility to develop psychiatric disorders(44). In this review, we highlight the role of the Netrin-1/DCC pathway in the development and adult plasticity of the PFC as a key mediator of depression in humans and stress vulnerability in mice. We hypothesize that alterations in the Netrin-1/DCC pathway prime the central nervous system to disruption by environmental factors such as stress. This renders individuals more susceptible to develop behavioral abnormalities.

II. THE NETRIN-1/DCC SIGNALING PATHWAY

Netrin-1 is a secreted protein that binds to cell surfaces and to the extracellular matrix to direct growth of DCC-expressing axons along an adhesive surface (haptotaxis)(27,28,45). It was described as a guidance cue attracting growing axons towards intended targets, where they can establish synaptic contacts(46), but subsequently was also identified as a repellent cue(47,48). The DCC:UNC5 ratio at the cell surface determines Netrin-1 signaling, leading to the recruitment of downstream proteins that induce cytoskeletal reorganization(49–51). Netrin-1, DCC, and UNC5 continue to be expressed in the adult brain and are involved in

the reorganization of neuronal structure and synaptic plasticity within local circuits (Figure 1B)(32,34,52) In this review we focus on the Netrin-1/DCC pathway.

A. DCC receptors

The *DCC* gene is on chromosome 18q and encodes a ~185 kDa protein (Figure 1A)(46). DCC receptors are expressed by selective neuronal populations, including cingulate 1, prelimbic and infralimbic sub-regions of the PFC and dopamine (DA) neurons in the VTA and substantia nigra pars compacta, across the lifespan and across species, including humans(53–56). Their levels decrease drastically from embryonic life and early adolescence to adulthood (Figure 1B)(32,34,52) and are regulated epigenetically via DNA methylation(57,58) and microRNAs (miRNAs)(42,59–62). For example, hypermethylation at the promoter site of the *DCC* gene leads to reduced DCC protein expression(57,58), whereas hypomethylation results in increased protein(64). In addition, two miRNAs, miR-218 and miR-9, have been shown to bind directly to the three prime untranslated region (3'UTR) of the *DCC* mRNA and decrease its expression in vitro and in vivo(42,59,61). DCC protein and miR-218 selectively colocalize in pyramidal neurons of human and mouse PFC and in VTA DA neurons, with their expression levels correlating negatively across postnatal development(42,59).

B. DCC signaling and maturation of the PFC

In humans, magnetic resonance studies have found that gray matter thickness in the PFC decreases across adolescence before stabilizing in adulthood, while white matter volume increases(14,63). This results from modifications occurring at the cellular level (e.g., neuronal architecture, synapse density, and neurotransmitter concentration)(65,66), and coincides with the acquisition of complex behavioral and cognitive abilities(67–69). In rodents, PFC maturation involves synaptic pruning, structural modifications of pyramidal neuron structure, DA axon ingrowth, and changes in expression/sensitivity of DA receptors(41,67,69–73) DCC receptors are highly expressed in cell bodies and dendrites of PFC pyramidal neurons and, to a lesser extent, GABAergic interneurons(56). Netrin-1 and DCC receptors facilitate cortical synaptogenesis by increasing the structural complexity of pyramidal neurons and by promoting axon–dendritic adhesive contacts(33). Both proteins are enriched at synapses where they regulate synaptic plasticity and trigger the potentiation of excitatory synaptic transmission via the insertion of GluA1 AMPARs into the postsynaptic membrane of pyramidal neurons in the adult forebrain(32,34). Whether and how altered levels of Netrin-1/DCC signaling in PFC pyramidal neurons within the fully differentiated adult brain lead to changes in their structure and functioning remains to be determined. Initial evidence suggests that such alterations might induce changes in dendritic spine morphology (74).

DCC receptors are also expressed by VTA DA neurons that innervate non-cortical targets, including the NAcc. Our work in mice shows that, in adolescence, the interaction between DCC receptors in mesolimbic DA axons, and Netrin-1 expressed by dendrites of NAcc neurons and located in the surrounding extracellular matrix, induces DA axons to recognize this region as their final target. Mesocortical DA axons lack or have scant levels of DCC receptors, and instead of recognizing the NAcc as their final target, they continue to grow

towards the PFC, including its orbital portion, across adolescence(41,71). By preventing mesolimbic DA axons from continuing to grow ectopically to the PFC, DCC receptors in DA neurons determine the structural and functional maturation of adult PFC pyramidal neurons(41,71). Amphetamine exposure during adolescence dysregulates DCC receptor expression in mesolimbic DA axons as well as Netrin-1 in the NAcc, profoundly disrupting PFC development and cognitive processing in rodents(40,43,75,76). These effects are restricted to recreational, but not therapeutic-like, doses of amphetamine, and are initiated by dysregulation of the *Dcc* repressor, miR-218(42,43). Whether stress in adolescence induces enduring alterations in PFC structure and function by targeting DCC receptors in DA neurons remains to be established.

III. RODENT MODELS OF STRESS SUSCEPTIBILITY

Depression is a heterogeneous disorder accompanied by a variety of symptoms, including recurrent episodes of sadness, hopelessness, lack of interest or pleasure (anhedonia), social avoidance, and suicidal ideation, among many others(2). Risk for MDD is mediated by the complex interaction between genetic and environmental factors, with the subjective nature of the depressive symptomatology representing a significant challenge for the understanding of its etiology, diagnosis, and treatment(77,78). Stressful life events, such as physical abuse, personal loss, or financial problems, are the main environmental risk factor for MDD in vulnerable individuals(79,80). This epidemiological observation has fostered the development of rodent stress models to induce behavioral alterations that resemble some of the core symptoms of human depression, including learned helplessness, anhedonia, reduced grooming, and changes in appetite, sleep, circadian rhythms, and social behavior(81–83). These models also recapitulate the molecular, cellular, and circuit signatures observed in MDD(2,83,84).

Rodent models of chronic stress involve the sustained or repeated activation of stress systems, including the hypothalamic-pituitary-adrenal (HPA) axis and many others, in which animals are subjected to a specific paradigm, including chronic administration of pharmacological activators of the stress response (e.g., corticosterone), chronic physical restraint, or chronic exposure to a range of physical stressors (e.g., footshock), over a period that ranges from 1 to 12 weeks. Chronic variable stress entails the application of several of these stressors to avoid the animals' habituation to a single type of stress. At the completion of the paradigms, animals are assessed in a battery of behavioral tests that measure phenotypic traits such as reward sensitivity, social interaction, sucrose preference, and time spent grooming (for an extensive review, see(81,82,85)). Stress-induced alterations in many of these behaviors can be reversed by chronic treatment with monoamine-based antidepressants which are typically used to treat depression in humans(81).

The chronic social defeat stress (CSDS) paradigm has more recently emerged as a validated mouse model that provides the advantage of segregating socially-defeated mice into susceptible and resilient populations based on a social interaction test (SIT) (86–88). The CSDS protocol involves an experimental mouse, usually from the C57BL/6J strain, being exposed to repeated bouts of social subordination (defeat) by an aggressive CD-1 mouse during several consecutive days(87). At the completion of the CSDS procedure, mice are

evaluated in the SIT to assess CSDS-induced social avoidance towards a social target (e.g., a novel CD-1 mouse)(87). Mice showing high social avoidance are classified as susceptible and show a range of other behavioral deficits, including reduced sucrose preference. Resilient mice do not exhibit most of these impairments, but instead display a series of behavioral and molecular adaptations that protect them from developing stress susceptibility. Although the CSDS protocol was originally standardized for male mice, novel protocols have been adapted for use in female mice, allowing the use of CSDS for studies on sex differences and vulnerability to stress(89–93).

IV. DCC RECEPTOR EXPRESSION IN THE PFC IS DYSREGULATED IN MDD AND DETERMINES SUSCEPTIBILITY TO CSDS

To understand the role of Netrin-1/DCC signaling pathway in depression, we have taken a translational approach using postmortem human brain tissue of adult subjects who were previously diagnosed with MDD and the CSDS mouse model. We measured *DCC* mRNA expression in the PFC of two separate and independent cohorts of depressed adult subjects who died by suicide and were antidepressant-free for at least three months before death. Depressed subjects exhibit significantly higher *DCC* mRNA levels (~50%) in comparison to psychiatrically healthy controls(39,59), which significantly and negatively correlated with a reduction in miR-218 expression (~50%)(59), suggesting miRNA mechanisms linking alterations in the Netrin-1/DCC pathway and MDD. Using the CSDS model, we found that adult mice that exhibit susceptibility to CSDS also display upregulation of *Dcc* mRNA and DCC protein in the PFC in comparison to control and resilient mice(59). Furthermore, susceptibility to CSDS is prevented by viral-mediated downregulation of *Dcc* in PFC pyramidal neurons, a manipulation that does not alter anxiety-like behaviors, learning, or locomotor activity(59). Adult exposure to CSDS does not lead to changes in *Dcc* mRNA levels in the VTA(59), a brain region highly associated with vulnerability to stress (86,88,94,96).

Our rodent studies further suggest that miR-218 in the PFC may act as a molecular switch of stress vulnerability because bidirectionally manipulating its expression leads to opposite CSDS-induced behavioral outcomes. Dampening miR-218 expression in the adult PFC using an antagomiR (antimiR-218) promotes susceptibility to a single exposure to social defeat(75). Furthermore, intranasal infusion of antimiR-218 turns resilient mice into susceptible, when assessed in a second SIT(75). In contrast, overexpression of miR-218 selectively in PFC pyramidal neurons protects against CSDS-induced social avoidance(75). These effects seem to involve DCC-dependent remodeling of dendritic structure because miR-218 overexpression in PFC pyramidal neurons of socially-defeated mice increases their dendritic spine density and prevents stress-induced reductions in thin spine density, most likely by promoting the formation of immature or nascent spines (Figure 2)(75). This finding is consistent with the role of DCC receptors in experience-dependent reorganization of synaptic connectivity in the adult brain(32,34,52).

Exposure to CSDS in adulthood may alter DCC expression in the forebrain regions. For example, a wholegenome bisulfite sequencing study of DNA methylation in the NAcc

revealed greater hypomethylated sites in non-CpG sequences of the *Dcc* gene body of susceptible mice, suggesting enhanced expression(95). An analysis of the two paralogs of miR-218(97), miR-218–1 and miR-218–2, showed no differential methylation within 20kb of miR-218–1, but identified two hypermethylated CpGs sites approximately 2kb downstream of miR-218–2, suggesting methylation mechanisms controlling both miR-218–2 and *Dcc* expression in this region.

V. GENETIC VARIANTS IN *NETRIN-1* AND *DCC* ARE ASSOCIATED WITH MDD

Twin studies and genome-wide association studies (GWAS) have estimated the heritability of MDD to range between ~30 to 40%(77,78). This heritability is thought to be highly complex with potentially many hundreds of genetic variations being involved, each having miniscule effects. Only a few genetic variants have been identified and consistently replicated in large human cohorts(78). Despite the relatively low heritability of MDD compared to other psychiatric disorders(78,98), increasing numbers of GWAS find that specific single-nucleotide polymorphisms (SNPs) within the *DCC* gene are linked to MDD or to traits associated with depression (Table 1).

One pioneer GWAS examined the interaction between genetic variants and environmental factors, such as social support and stressful life events, in a small sample of African-American and Hispanic/Latino women. There were no SNPs that achieved genome-wide significance, but an intron variant within the *DCC* gene, rs4542757, had the strongest association signal with MDD(99). Subsequently, a GWAS comprising non–hypothesis-driven pathway analysis and regional heritability analyses was performed to identify gene networks that were previously linked to MDD in two independent and large human cohorts(100–102) The polygenic risk score (PRS) found the Netrin-1 signaling pathway as the pathway most consistently associated with MDD(102). Closer analysis revealed that variants within the *DCC* gene region encoding Netrin-1 binding sites had the greatest heritability (Figure 1A)(102).

A follow-up study(103) assessed the white matter integrity of individuals with high PRS for MDD derived from the Netrin-1 signaling pathway identified previously(102). PRSs for the Netrin-1 pathway were associated with alterations in the superior longitudinal fasciculus, a fiber tract that connects the frontal, temporal, and parietal lobes(103). Another study that combined genetic analysis and functional brain imaging reported a total of 11 novel loci highly correlated with anhedonia and MDD, and, to a lesser degree, with schizophrenia and bipolar disorder. The most significant hit was at the *DCC* locus on chromosome 18. Anhedonia was associated with smaller total grey matter volume, and reduced white matter integrity in the NAcc, caudate, and PFC(106). These GWAS are consistent with our findings showing that humans who develop with *DCC* haploinsufficiency exhibit decreased striatal volume and decreased mesocorticolimbic anatomical connectivity(104).

Polymorphisms within the *DCC* and *NETRIN-1* genes have also been linked to psychological traits related to MDD, including neuroticism, subjective well-being, or anhedonia (Table 1). For example, the intronic variants of the *DCC* gene, rs62100776(105)

and rs11663393(107) are associated with depressive symptoms, in large GWAS meta-analyses, whereas the *DCC* SNPs, rs8084280 and rs62099230, localized within the region encoding the receptor binding sites, are linked to mood instability and suicidality(108,110). In addition, the *NETRIN-1* SNP, rs8081460, is associated with neuroticism; however, this SNP effect has not been replicated in two independent smaller samples(112). Finally, a study conducting gene-based tests identified *DCC* among six genes associated with depressive symptoms in UK Biobank participants(109).

Consistent with our postmortem results, a blood microarray analysis of two independent case-control studies identified *overexpression* of the *DCC* mRNA, among 165 differentially expressed genes in MDD(111) and a methylome-wide CpG-SNP analysis found reduced methylation within the *DCC* gene in blood samples of MDD patients(113). Altered methylation of the *Netrin-1* gene has also been associated with depression in a study with monozygotic twins with and without a lifetime history of early-onset depression(114), suggesting that variations in the methylation levels of these genes may add to what can be explained by the sequence variation.

The convergence between human postmortem studies, rodent studies, and GWAS suggests that the relevance of the Netrin1/DCC pathway in the etiology of MDD results from its developmental role in the spatiotemporal organization of circuitries involved in cognition and emotion. Perhaps it is not surprising that the largest genome-wide meta-analysis of psychiatric disorders conducted to date, with more than 725,000 cases-controls, across eight disorders, found the intronic *DCC* SNP, rs8084351, to have the most significant and pleiotropic effect(115). Overall, the identified SNPs are present within regions important for the function of the DCC protein or within intronic regions, likely leading to altered *DCC* gene transcription, possibilities that now warrant direct investigation. The link between alterations in this pathway and MDD may occur in the absence of stress but may be exacerbated by adverse experiences, including those occurring in adulthood, as our studies in rodents indicate(59,75). DCC protein expression decreases from early life to adulthood(33,42,54); stress-induced alterations in the Netrin1/DCC pathway seem to have different outcomes depending on the developmental stage when this is experienced (Figure 2 and Figure 3).

VI. BIOMARKERS OF VULNERABILITY TO MDD: POTENTIAL ROLE OF MIR-218

Biomarkers can provide information about traits that could predict the onset and course of a disease, confirm the clinical status of an individual, and be used to track the effectiveness of a treatment regimen. Biomarkers range from the blood pressure to complex biochemical analysis of blood and other peripheral tissues(116,117). Genetic, behavioral, and neuroimaging indices have also been introduced as potential biomarkers of neuropsychiatric disorders(118–120), but to date there is no bona fide biomarker for any psychiatric syndrome that is used clinically to help guide diagnosis, treatment, or prognosis.

The use of miRNAs as non-invasive biomarkers of depression and potential mediators of pharmacological and behavioral interventions is gaining interest(121,123). miRNAs can be

accessed via bodily fluids, are highly stable due to their biological packaging, and may represent a signature of alterations occurring in the brain(123,125). Interestingly, given the diverse etiology of MDD, microarrays or small RNA sequencing studies have identified a few miRNAs as potential biomarkers of MDD, including miR-1202, miR-135, miR-146a/b-5, miR-425–3p, miR-24–3p, miR-941, and miR-589 (for reviews see: (117,121,123), which have also been found to be altered in postmortem brain tissue and plasma or blood-derived cells of MDD subjects(122,124,126–129)). These correlational studies, however, do not provide direct evidence that brain miRNA alterations can be detected in blood and that miRNAs are functionally implicated in MDD vulnerability.

To begin filling this gap, we showed that circulating miR-218 levels may serve as a biomarker of stress vulnerability through a mechanism that involves the PFC. Adult susceptible mice to CSDS exhibit reduced blood levels of miR-218, and these levels correlate with stress-induced social avoidance(75). These findings are consistent with a recent small RNA sequencing study reporting miR-218 as one of the miRNAs differentially downregulated in blood obtained from MDD patients(130) and with our report that miR-218 is decreased in the PFC of MDD individuals and of CSDS-induced susceptible mice(75). We further showed that experimentally-induced miR-218 downregulation in the PFC associates with a parallel reduction in circulating levels of miR-218 in mice. Conversely, viral-mediated upregulation of miR-218 selectively in PFC pyramidal neurons causes increased miR-218 levels in blood(75).

Circulating miRNAs exist in high-density lipoproteins and exosomes that prevent them from degradation before they reach target cells(131,132). Recent evidence demonstrates that motor neurons can release miR-218 to the extracellular space to be taken up by astrocytes(133). Our findings(75) raise the possibility that PFC pyramidal neurons may release exosomes containing miR-218, reaching the peripheral circulation. It is necessary to determine whether viral-mediated manipulations of miR-218 in brain regions other than the PFC, including the HPC, would also result in measurable changes of this miRNA in blood. Future experiments are required to definitively establish this novel mechanism as well as the potential of miR-218, and presumably other miRNAs, to serve as validated biomarkers for aspects of MDD.

VII. CONCLUSIONS AND FUTURE DIRECTIONS

A clear understanding of the pathophysiology of MDD becomes urgently necessary for developing novel preventive strategies and generating evidence-based and rapid-acting therapeutic interventions. This review creates a translational bridge between human and rodent research that points towards Netrin-1 and its DCC receptor as important contributors to the pathophysiology of MDD and opens a new venue for future studies. From the preclinical side, we report studies showing that alterations in DCC protein expression induced by adult stress confer vulnerability to depression-like behaviors. From the human side, we highlight evidence from GWAS showing that *DCC* polymorphisms not only are associated with depressive symptoms but also with alterations in brain circuitry. We propose a role for miR-218, readily measured in blood, as a potential biomarker of stress

vulnerability. The consistency between studies is striking, considering the heterogeneous methodologies and samples in humans and mice.

Adolescent and adult women are twice as likely as men to develop MDD and other stress-related disorders. They begin to manifest depressive symptoms early in adolescence and continue to be at heightened risk throughout life. Yet, the great majority of preclinical studies have been conducted primarily on males, neglecting important aspects of sex differences, such as sex hormones, chromosomal differences, behavioral coping strategies, and stress reactivity. There is a need to study the role of *Dcc* and miR-218 in female mice exposed to chronic stress during adolescence and adulthood following protocols that are now widely validated. GWAS should also examine the specific interaction between *DCC* polymorphisms and sex in large and independent cohorts of men and women suffering from MDD(99).

Biomarkers can be observed at baseline conditions to predict the outcome of an illness or response to a treatment (*predictors*), or can change over the course of an illness or as the result of a specific treatment (*mediators*)(134). Our results show that mice that become susceptible to CSDS display low levels of circulating miR-218(59). Whether circulating miR-218 can predict stress vulnerability or the outcome of antidepressant treatment remains to be answered. Our preliminary data suggest that circulating miR-218 during adolescence predicts susceptibility to adult exposure to CSDS (135). miR-218 levels in blood exhibit a very interesting developmental pattern that mirrors PFC miR-218 expression, and our data demonstrate that primary changes in miR-218 expression in PFC neurons per se can cause parallel changes in circulating levels of this miRNA(75). Future studies will provide an understanding of the potential role of circulating miR-218 as a developmental predictor and mediator of stress vulnerability. Targeting miR-218 or directly the Netrin-1/DCC pathway in early life (e.g., during adolescence) may be a promising venue for the development of prevention and intervention strategies.

Perinatal stress, including prenatal and early stress, increases the risk for depression and other stress-related disorders later in life by two- to four-fold(136). This effect may be mediated by changes in the Netrin/DCC signaling pathway. For example, *Dcc* and *Unc5c* mRNA were among 916 transcripts differentially expressed in the HPC of adult rats exposed to prenatal stress(137). We propose that stress across the lifespan dysregulate the Netrin-1 system in selective PFC-limbic circuits, via epigenetic mechanisms. In this context, the alterations in *DCC* and miR218 expression observed in the adult MDD postmortem brain studies may already be present earlier in development and/or result from early life adversity. Longitudinal studies collecting peripheral tissue samples may be able to address this important issue(111).

Our work on the adolescent development of the mesocorticolimbic system shows that DCC signaling within DA neurons of the VTA determines the extent of their innervation to the PFC and the organization and function of the PFC local circuits in adulthood(71). This effect is mediated by miR-218 regulation of *Dcc* in the VTA(42) and can be modified by exposure to drugs of abuse during adolescence(40,71,76,138,139). Whether chronic stress in adolescence regulates DCC receptor and miR-218 expression in the VTA, disrupting the

development of the DA innervation to the PFC, and whether this effect is sex-specific, needs to be determined. We hypothesize that stress in adolescence alters DCC expression in mesolimbic DA axons, triggering their mistargeting in the NAcc and their ectopic growth to the PFC, remodeling adult PFC synaptic circuitry (Figure 3).

In conclusion, the work summarized here demonstrates the importance of the Netrin-1/DCC signaling pathway in controlling susceptibility to chronic stress and the development of MDD, with converging evidence obtained from humans and mouse models. The work underscores the importance of unbiased approaches with both species in identifying novel pathogenic mechanisms of MDD.

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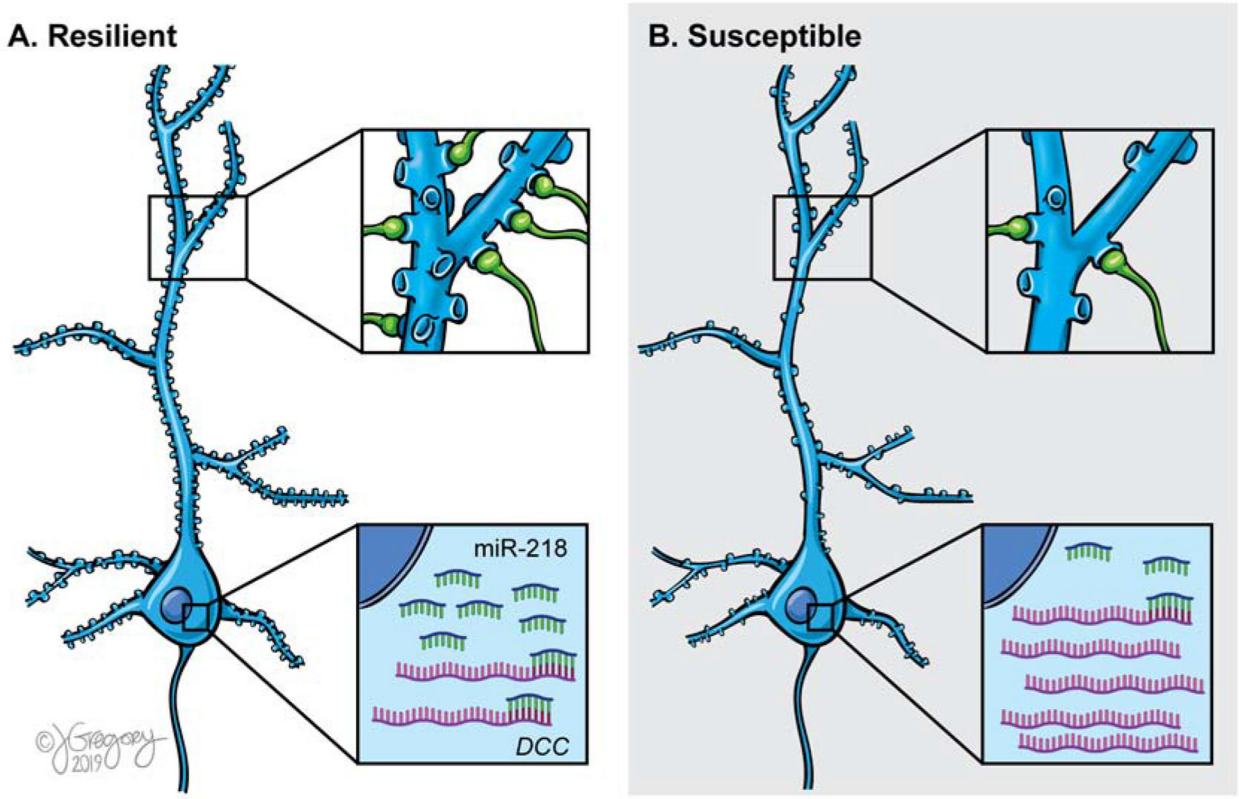

Figure 1. The structure and function of Netrin-1 and its receptor DCC.

(A) Netrin-1 is a member of the laminin superfamily and is a secreted protein associated with the extracellular matrix. Its terminal laminin VI domain is globular and is followed by domain V, which is composed of three epidermal growth factor (EGF) repeats. The carboxy-terminal, C-domain (called NTR), is enriched in basic amino acids and binds heparin in the extracellular matrix through interactions with heparin sulfate proteoglycans. The VI and V domains are responsible for the binding of receptors. The DCC receptor is a single pass transmembrane protein, with an extracellular fragment composed of four immunoglobulin-like (Ig-like) domains folding into a horseshoe conformation, and six fibronectin type III (FN) domains where Netrin-1 binds to FN4 and FN5 domains. The cytosolic portion of DCC contains three domains, P1, P2 and P3, responsible for signaling. Netrin-1 has 3 binding sites for DCC, two of these sites can be replaced by other Netrin-1 receptors, including UNC5. Upon Netrin-1 binding, DCC receptor molecules form a signaling complex by interacting through their cytosolic P3 domains. This initiates the recruitment of intracellular components, including members of the NCK family of adaptor proteins and focal adhesion kinases, which in turn activate Src family kinases, Rho GTPases, the release of Ca2+, protein translation and reorganization of the actin cytoskeleton. The UNC5 receptor is a transmembrane protein with two Ig-like domains (most likely responsible for Netrin-1 binding), two thrombospondin domains (Tsp), and a large cytoplasmic tail composed of three domains, a ZO-1/Unc5 (ZU-5) domain, a DCC binding motif (DB), and a death domain (DD). When binding to Netrin-1, UNC5 and DCC proteins form receptor complexes by interacting through DCC's cytosolic P1 domain and UNC5's ZU5 and DB domains. **(B)**

DCC-mediated Netrin-1 signaling influences the growth, targeting, and arborization of axons, dendritic growth, and synapse formation. These functions are restricted or predominant to specific periods of development and neuronal circuits. Netrin-1 and DCC continue to be expressed in the adult brain and are involved in refining neuronal structural organization and synaptic plasticity. Netrin-1 and DCC expression decrease from early life to adulthood. This switch in expression levels presumably coincides with the transition of their role as organizers of the connectivity of large neuronal networks to the refinement of established local circuits. DCC-mediated Netrin-1 signaling is thought to mediate predominantly neuron-neuron connectivity, although these proteins are expressed in other cell types, including glia, depending on the CNS region.

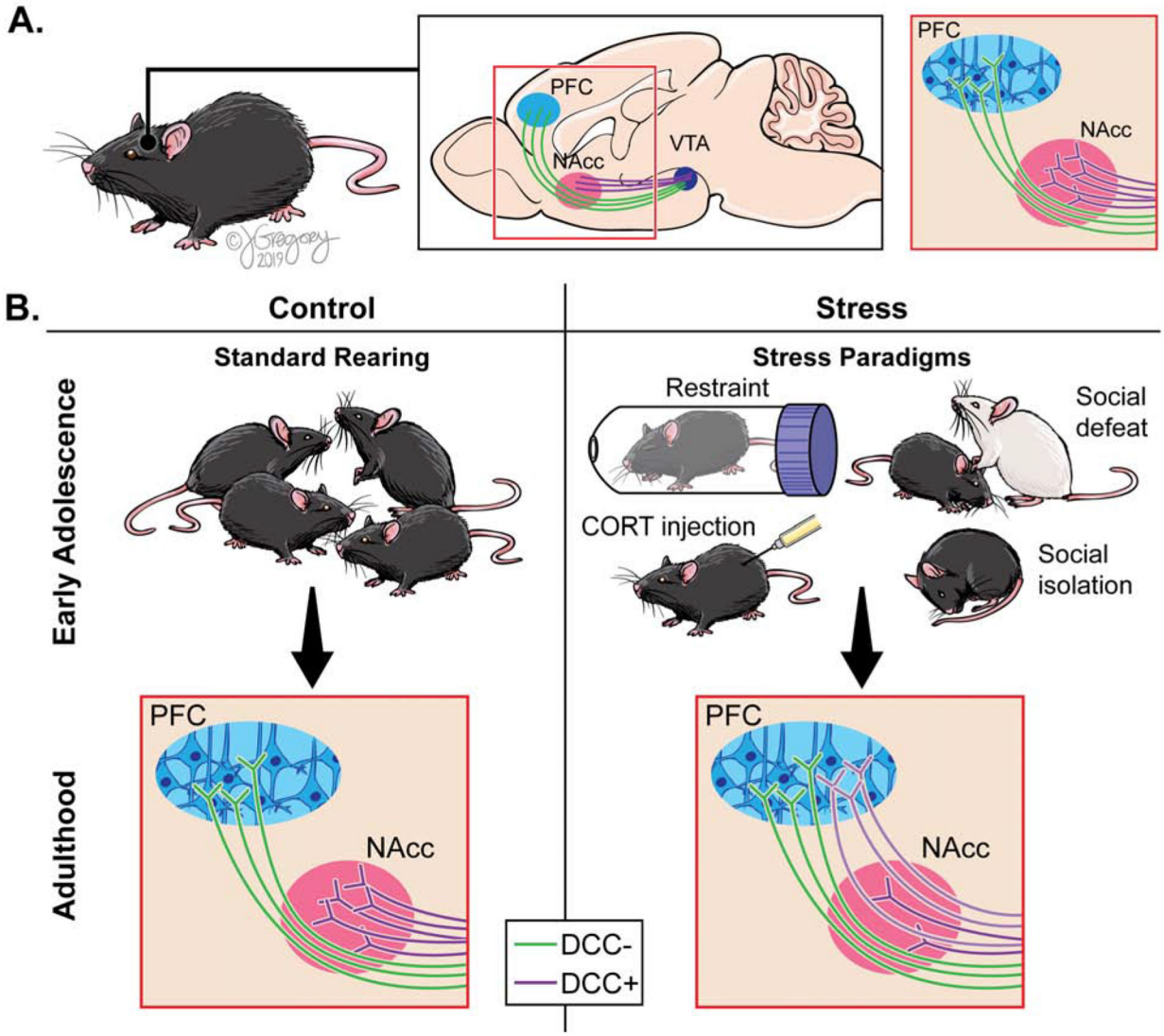

Figure 2. miR-218 levels in the PFC determine susceptibility versus resilience to chronic stress in adulthood.

(A) Experimentally-induced increased miR-218 expression in adult PFC pyramidal neurons prevents stress-induced *DCC* upregulation, maintaining dendritic structure and optimal synaptic connectivity. **(B)** Stress-induced miR-218 downregulation in adult PFC pyramidal neurons results in *DCC* upregulation, causing dendritic structure modifications and aberrant synaptic connectivity.

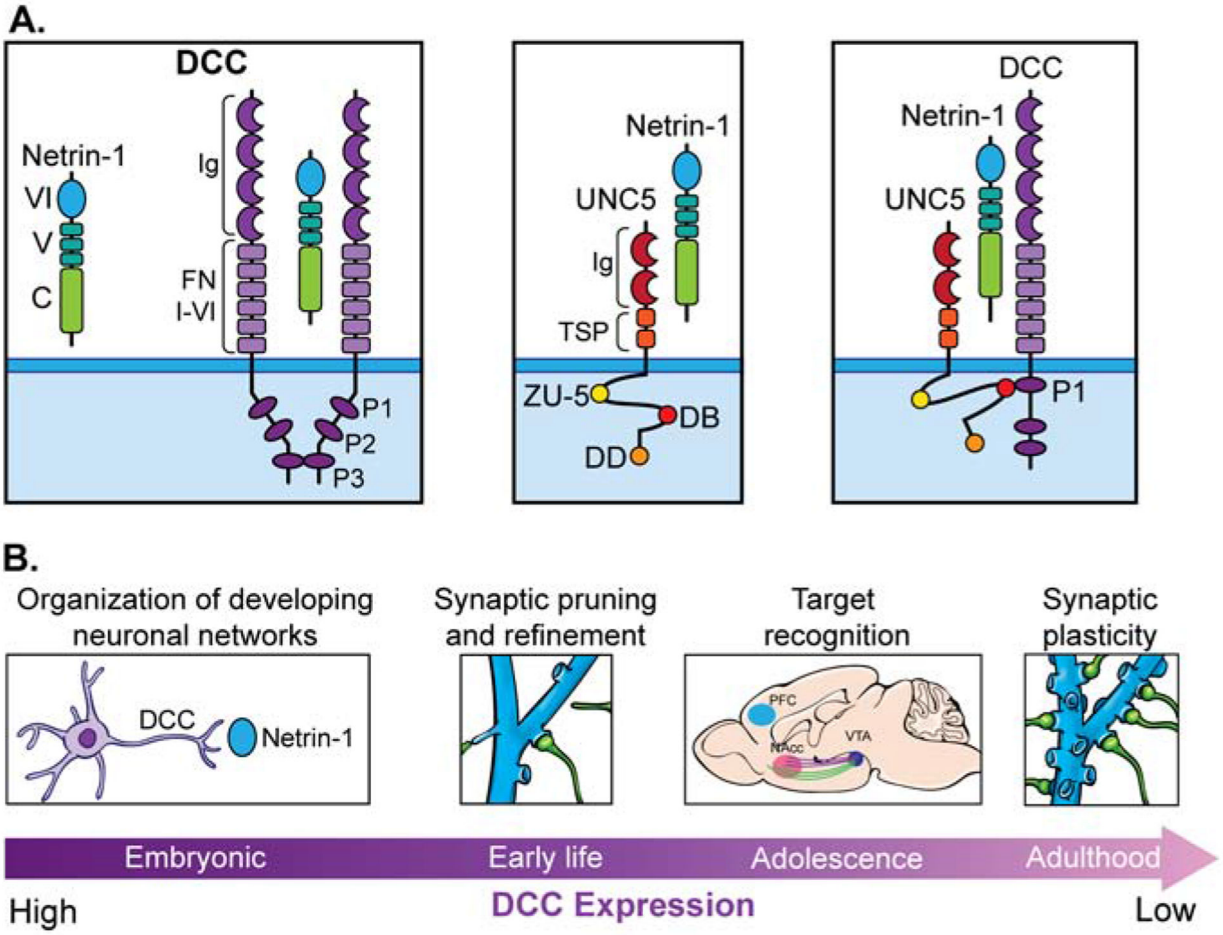

Figure 3. Stress in adolescence may alter ongoing PFC development by disrupting DCC expression in dopamine axons.

(A) Sagittal representation of the adult mesocorticolimbic system where DA axons expressing DCC receptors (in purple) innervate the NAcc, whereas DA axons that lack DCC receptors (in green) innervate the PFC. **(B)** Stress in adolescence may alter the topographic organization of mesolimbic and mesocortical DA axons in adulthood. For example, stress may reduce DCC expression in DA axons that normally recognize the NAcc as their final target, inducing their ectopic growth to the PFC.

Table 1.

Summary of published GWAS linking single nucleotide polymorphisms within the NETRIN1/DCC signaling pathway with MDD, Depressive Symptoms or Depression-associated traits. Human genes are designated in italicized uppercase letters.

Author	Year	Total N	Cases	Controls	Replication Sample Cases	Replication Sample Control	Meta-analysis sample	Ancestry	MDD DEFINITION	GWAS hits	Putative Genes DCC or Netrin	SNP - location	Functional consequence	Notes
Okbay et al. (105)	2016	75306	16471	58835	105739			E	Frequency, in the past two-weeks, in which the respondent experienced feelings of unenthusiasm or disinterest and feelings of depression or hopelessness.	2	***DCC***	**rs62100776**	intron variant	
Smith et al. (112)	2016	91370 (47196 ♀ / 44174 ♂)					15346	E	Eysenck Personality Questionnaire-Revised (EPQ R-S) Short Form's Neuroticism scale	9				**Netrin-1 SNP, rs8081460**, was associated with neuroticism in the UK Biobank sample. This SNP effect did not replicate in independent samples
Zeng et al. (102)	2017	6455	1123	5332	9240	9519		E	Structured Clinical Interview	1	***Netrin 1***			**Polygenic risk scores (PRSs) calculated for netrin-1 pathway more accurately predicted MDD** in one of the cohorts compared with PRS calculated for the whole genome
Dunn et al. (99)	2016	0	7179 AA, 3138 Hs					AA, Hs	Center for Epidemiological Studies of Depression Scale (CES-D)	0				The top signals in AA were rs73531535 (located 20kb from GPR139, p=5.75×10–8) and rs75407252 (intronic to CACNA2D3, p=6.99×10–7). In Hs, the top signals

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														were rs2532087 (located 27kb from CD38, p=2.44×10–7) and rs4542757 (intronic to DCC, p=7.31×10–7).
Ward et al. (108)	2017	113968	53525	60443					Mood instability measured by a single question: 'Does your mood often goes up and down?	4	***DCC***	**rs8084280**	intron variant	
Wray et al. (107)	2018	480359	135458	344901				E	Diagnostic Interview. Cases were required to meet international consensus criteria (DSM-IV, ICD-9, or ICD-10	44	***DCC***	**rs11663393**	intron variant	
Amare et al. (140)	2019	336753	90150	246603	NR	NR		E Ch ♀	Self-reported MDD (srMDD), recurrent MDD(rMDD),	1				rMDD significant SNP was not replicated in the replication sample
Howard et al. (141)	2019	807553	246363	561190	414055			E	Clinical interview and broader criteria	102	***DCC***	**rs7227069**	intron variant	
Arnau-Soler et al. (109)	2019	4919 (2990 ♀ / 1929 ♂)			99057			E/Sc	Questionnaire and psychological assessment	0				Post-GWAS gene-based test identified six genes assocaited with the Patient Health Questionnaire validated to screen mental illness: DCC, ACSS3, DRD2, STAG1, FOXP2 and KYNU
Barbu et al. (103)	2019	6420						E		0				**PRS from the Netrin-1** signaling pathway is significantly and specifically associated with

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														white matter integrity in thalamic radiations, namely lower fractional anisotropy, and higher mean diffusivity
Roberson-Nay et al. (114)	2018	150 pairs of MZ twins						E	DSM-V criteria for MD	0	*0*	0		Identified **altered methylation in Netrin-1**. Gene enrichment analyses implicated genes related to neuron structures and neurodevelopmental processes including cell-cell adhesion genes (e.g., CDHs, PCDHAs, PCDHA1C/2C). Genes previously implicated in mood and psychiatric disorders as well as chronic stress (e.g., HDAC4, NRG1)
Strawbridge et al.(110)	2019	122822	39265	83557	15735 & 23923	84499 & 84167		E	Questionnaire to assess suicidality phenotype	3				The gene-based analysis was used to identify genes containing potential composite association signals that were not identified by the individual SNP analysis, but which might nevertheless contribute to biological mechanisms underlying suicidality. The gene-based analysis highlighted CNTN5, ADCK3/ COQ8A, CEP57, and FAM76B and **DCC** for suicidality. EIF4A1

Author	Year	Total N	Cases	Controls	Replication Sample Cases	Replication Sample Control	Meta-analysis sample	Ancestry	MDD DEFINITION	GWAS hits	Putative Genes DCC or Netrin	SNP - location	Functional consequence	Notes
														and SENP3 and **DCC** for deliberate self-harm
Ward et al. (106)	2019	375275							Anhedonia	11	***DCC***	**rs72923287**	intron variant	PRS for anhedonia was associated with poorer brain white matter integrity, smaller total grey matter volume, and smaller volumes of brain regions linked to reward and pleasure processing, including nucleus accumbens, caudate, and medial frontal cortex. A locus in the **DCC** gene was the most significant hit associated with anhedonia
Lee et al. (115)	2019	727126	232964	494162				E	Cross psychiatric disorders	23 associated with at least four of the disorders	***DCC***	**rs8084351**	intron variant	The region surrounding SNP rs8084351 at the gene DCC featured the most pleiotropic association. This region showed association with all eight psychiatric dissordes studied (MD, SCZ, BIP, ADHD, ASD, TS, ANO, OCD)

E=european

Sc=Scottish

rMDD=recurrent major depression

ASD=Autism spectrum disorder

AA= African American

BIP=Bipolar Disorder

MD= Major Depression

TS=tourette syndrome

Hs: Hispanic

TS= two samples

SCZ= Schizophrenia

ANO=anorexia nervosa

Ch= Chinese

PRS=Poygenetic Risk Score

ADHD= Attention deficit hyperactivity disorder

OCD=Obsessive–compulsive disorder