Cognitive dysfunction, including significant impairments in learning, behavior, and attention, is found in over 10% of children in the general population. However, in the common inherited cancer predisposition syndrome, neurofibromatosis type 1 (NF1), the prevalence of these cognitive deficits approaches 70%. As a monogenic disorder, NF1 provides a unique genetic tool to identify and dissect mechanistically the molecular and cellular bases underlying cognitive dysfunction. In this review, we discuss NF1 fly and mouse systems that mimic many of the cognitive abnormalities seen in children with NF1. Further, we describe discoveries from these models that have uncovered defects in the regulation of Ras activity, cAMP generation, and dopamine homeostasis as key mechanisms important for cognitive dysfunction in children with NF1.

Introduction
NF1 is one of the most common neurogenetic disorders affecting the nervous system. The hallmark of NF1 is the development of tumors involving the central and peripheral nervous systems. In this condition, affected individuals are prone to the formation of peripheral nerve sheath tumors (i.e., neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors) and brain tumors (optic pathway gliomas and malignant gliomas). Moreover, 50–70% of children with NF1 manifest specific cognitive impairments, including difficulties with attention, executive function, language, visual perception, and learning [1–4]. Although most children exhibit some form of cognitive deficit that negatively impacts upon their scholastic performance, the specific cognitive abnormality present (i.e., attention deficit, spatial memory impairment, fine motor delay) and the severity of the deficit varies greatly from child to child.

In concert with clinical studies characterizing the spectrum of learning, behavioral, and motor delays in children with NF1, laboratory investigations have begun to define the molecular and cellular etiologies for these common problems. Using NF1 genetically engineered strains of mice and flies, investigators have successfully modeled many of the cognitive and behavioral deficits seen in children with NF1, and employed these model systems to better define the role of the NF1 protein (neurofibromin) in normal central nervous system (CNS) neuronal function. This review will highlight the basic neurobiological insights that have derived from the use of these robust preclinical strains as well as their importance for the identification and validation of new therapeutic drug targets relevant to the treatment of children with NF1.

Clinical features of NF1
NF1 is a common nervous system disorder, affecting 1 in 3500 people globally [5]. NF1 is inherited in an autosomal dominant manner, although ~50% of individuals present with de novo mutations, and represent the first member of their family with NF1 [6]. Although NF1 genetic testing is available for select individuals, the diagnosis of NF1 is most often established on clinical grounds (Table 1). To be given the diagnosis of NF1, individuals must have at least two features of the condition, including greater than five café-au-lait macules (birthmarks), skinfold (underarm or groin) freckling, Lisch nodules (irish hamartomas), neurofibromas, an optic pathway glioma, a distinctive bone abnormality (i.e., tibial dysplasia), or a first degree relative with NF1 [7]. In addition to these features, individuals with NF1 may also manifest learning/behavioral problems, malignant gliomas, T2 hyperintensities on neuroimaging (i.e., magnetic resonance imaging) studies, enlarged heads (macrocephaly), gross and fine motor delays, short stature, and other less common cancers [8–13].

Cognitive and Behavioral Deficits in Children with NF1
Cognitive problems are the most frequently observed neurological impairments in children with NF1. The majority of children display some degree of cognitive deficits [1], which limit their full academic achievement and overall quality of life. Clinical studies examining cognitive problems in NF1 have revealed a left shift in average IQ, ranging from low to normal IQs, with specific learning deficits being observed in 30–70% of children [1,14,15]. Additionally, children with NF1 exhibit poor performance on tasks of reading, spelling, and mathematics, impaired expressive and receptive language skills, deficits in visuospatial and visuoperceptual skills, and defects in executive function (planning and concept formation) [1,16,17]. Although less common, there is also an increased incidence of autistic spectrum disorder in children with NF1 [18]. Problems with attention and behavior in children with NF1 can also negatively affect school performance and social interactions [19–21]. Nearly 70% of children with NF1 report deficits in one or more of the attention system...
Table 1. NF1 clinical manifestations

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Diagnostic criteria (fulfillment of ≥2)</th>
<th>Prevalence (% of NF1 population)</th>
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<tbody>
<tr>
<td>Family history</td>
<td></td>
<td>50%</td>
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<tr>
<td>Neurocutaneous</td>
<td></td>
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<tr>
<td>Café-au-lait spots</td>
<td>6 or more</td>
<td>99% in adults</td>
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<tr>
<td>Axillary/inguinal freckling</td>
<td>2 or more</td>
<td>85%</td>
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<tr>
<td>Lisch nodules</td>
<td>2 or more</td>
<td>95% in adults</td>
</tr>
<tr>
<td>Neurofibromas</td>
<td>2 or more</td>
<td>99% in adults</td>
</tr>
<tr>
<td>Plexiform neurofibromas</td>
<td>1 or more</td>
<td>20–45%</td>
</tr>
<tr>
<td>Skeletal</td>
<td></td>
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<tr>
<td>Scoliosis</td>
<td>ND</td>
<td>5–10%</td>
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<tr>
<td>Bone dysplasia</td>
<td>ND</td>
<td>1–5%</td>
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<tr>
<td>Pseudarthrosis</td>
<td>ND</td>
<td>2%</td>
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<tr>
<td>Short stature</td>
<td>ND</td>
<td>30%</td>
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<tr>
<td>Macrocephaly</td>
<td>ND</td>
<td>45%</td>
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<tr>
<td>Tumors</td>
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<tr>
<td>Optic pathway glioma</td>
<td>ND</td>
<td>15%</td>
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<tr>
<td>Malignant peripheral nerve sheath tumor</td>
<td>ND</td>
<td>2–3%</td>
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<tr>
<td>Low-grade glioma</td>
<td>ND</td>
<td>2–3%</td>
</tr>
<tr>
<td>Malignant glioma</td>
<td>ND</td>
<td>1–2%</td>
</tr>
<tr>
<td>Optic pathway glioma</td>
<td>ND</td>
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</table>

*ND, non-diagnostic; denotes NF1 clinical features associated with disease, but are not assessed for diagnosis.

domains (sustained, selective, divided, and shifting attention) [1,22,23], and one-third to one-half of children are diagnosed with attention-deficit hyperactivity disorder (ADHD) [1,2]. Moreover, children with NF1 tend to be impulsive, and often have difficulty detecting and responding to social cues.

Neurofibromin structure and function

The NF1 gene resides on chromosome 17 [5,24] and encodes a large cytoplasmic protein (neurofibromin), encompassing 2818 amino acids and over 60 exons. Neurofibromin contains several domains, including a cysteine-rich domain (CSRD), a leucine repeat domain (LRD), and a Ras-GAP domain (GRD) (Figure 1a). The NF1 gene also contains at least 3 alternatively spliced exons, 9a, 23a, and 48a, each with unique properties. Exon 9a-containing neurofibromin is a neuron-specific isoform [25,26], whereas exon 48a-containing neurofibromin is expressed in muscle [27]. Exon 23a neurofibromin interrupts the normal function of the GAP domain, but has a more widespread tissue distribution [28–30]. Interestingly, mice engineered to lack exon 23a do not have an increased tumor predisposition, but manifest specific learning impairments [28]. Current studies are focused on defining the functional consequences of NF1 alternative splicing, especially exon 9a, on neurofibromin signaling and NF1-associated tumor and cognitive deficits.

![Figure 1. Neurofibromin structure and function. (a) Neurofibromin is a 2818 amino acid protein that contains multiple alternatively spliced exons (9a, 23a, and 48a shown as red bars) and encodes several distinct functional domains, including a cysteine-rich domain (CSRD), a Ras-GAP domain (GRD), and a leucine-repe...](image-url)
The neurofibromin GRD functions in a similar fashion to other GTPase-activating proteins (GAPs), which negatively regulate the activity of the p21-Ras proto-oncogene (Figure 1b). Ras is recruited to the plasma membrane by adaptor proteins and is activated by receptor tyrosine kinases (RTKs) following growth factor binding. At the membrane, guanine nucleotide exchange factors (GEFs) enable Ras to bind GTP to become active and transmit growth-promoting signals. In its active state, Ras signals to several downstream effectors, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3 kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling pathways. Neurofibromin acts as an inhibitor of Ras activity by catalyzing the hydrolysis of active GTP-bound Ras to inactive GDP-bound Ras, such that in cells lacking neurofibromin, there are high levels of Ras pathway (i.e., MEK/FAK and PI3K/mTOR) activity and increased cell growth [31–34]. In addition to Ras regulation, neurofibromin is also a positive regulator of intracellular cAMP levels. Studies initially performed in Nf1 mutant flies revealed that reduced Drosophila larval size did not result from deregulated Ras signaling, but rather was dependent on cAMP-mediated protein kinase A (PKA) activity [35,36]. Subsequent studies in mice likewise demonstrated that both Nf1-deficient embryonic brains and postnatal astrocyte cultures had reduced cAMP levels [37,38]. Although the precise mechanism(s) underlying neurofibromin control of cAMP homeostasis has yet to be fully elucidated, there appear to be both Ras-dependent and Ras-independent modes of regulation [36,37,39].

**NF1 models of learning, memory, and attention deficits**

Inspection of the predicted protein sequence of the *Drosophila* and murine Nf1 homologs reveals 60% and greater than 98% identity to the human NF1 gene product, respectively, supporting the use of Nf1 mutant mouse and fly models to study many of the cognitive and behavioral features seen in the human condition [35,40,41]. In this regard, robust mouse and fly models have been developed and employed for preclinical discovery and validation initiatives aimed at improving clinical outcomes for children and adults with NF1 (Figure 1c).

**The role of Ras activation in learning and memory**

Studies pioneered in the late 1990s by Alcino Silva and colleagues employed mice harboring a germline inactivating mutation in one Nf1 allele. These Nf1 heterozygous (Nf1+/−) mice are genetically similar to children with NF1 who start life with one functional and one non-functional copy of the Nf1 gene in every cell. Nf1+/− mice exhibit impaired spatial learning in the Morris water maze, a behavioral task used to assess hippocampal-based learning [42,43]. These learning defects improve with training time, supporting clinical observations that children with NF1 can learn with additional focused training and resources [3]. Concomitant with defects in spatial learning, Nf1+/− mice also have decreased CA1 hippocampal long-term potentiation (LTP) following theta-burst stimulation [43]. Further characterization of this LTP deficit revealed abnormalities in both early-phase and long-term LTP maintenance [43,44]. Whereas early-phase LTP is thought to underlie immediate learning, late-phase LTP regulates long-term memory formation [45]. In this manner, abnormalities in both early-phase and late-phase LTP could contribute to the learning and memory deficits in Nf1+/− mice.

To define better the connection between reduced neurofibromin and impaired hippocampal function, Silva and colleagues explored the possibility that increased Ras activity was responsible for the observed cognitive deficits [43]. Their demonstration of increased Ras and MEK activity in the brains of Nf1+/− mice prompted genetic rescue experiments, where Nf1+/− mice were crossed with mice to reduce Ras (K-Ras and N-Ras) expression (Nf1+/−; K-Ras<sup>−/−</sup> or Nf1+/−; N-Ras<sup>−/−</sup> mice). In these studies, performance of Nf1+/− mice with either reduced K-Ras or N-Ras expression was comparable to that found in wild type control mice [43]. Similarly, pharmacological inhibition of Ras using a drug that blocks the post-translational farnesylation of Ras (Lovastatin) improved performance of Nf1+/− mice on hippocampal-based spatial learning tests [43,46]. Collectively, these findings provide strong evidence for a functional link between reduced brain neurofibromin expression, elevated Ras activity, and cognitive deficits, and prompted clinical trials using Lovastatin-like treatments for children with NF1 [47].

The cellular and neurochemical bases for impaired LTP and learning in Nf1+/− mice have also been investigated. Using Nf1 conditional knockout (CKO) mice in which one copy of the Nf1 gene was selectively inactivated in neurons or astrocytes, the consequence of reduced neurofibromin expression in distinct cell populations on mouse learning and memory was evaluated [48]. Although reduced Nf1 expression in neurons recapitulated the learning and memory deficits observed in Nf1+/− mice, reduced Nf1 expression in glial fibrially acidic protein (GFAP)-positive astrocytes had no effect on learning or memory. Neuron subpopulation-specific Cre driver lines have also been employed to address learning deficits after selective Nf1 inactivation. Specifically, reduced Nf1 expression in excitatory and inhibitory neurons (synapsin-I–Cre mice) or GABA-containing neurons [distal-less homeobox 5/6 (Dlx5/6)–Cre mice], but not forebrain pyramidal neurons [i.e., Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CAMKII)–Cre mice], resulted in defects in learning and memory similar to those observed in Nf1+/− mice. These observations demonstrate a primary neuronal defect as a responsible cellular etiology underlying murine Nf1 learning and memory dysfunction.

Based on the finding that inhibitory neurons were sensitive to the effects of reduced Nf1 gene expression, it was subsequently shown that Nf1+/− mice had increased hippocampal GABA inhibitory tone. In these studies, elevated MAPK-mediated synapsin-I phosphorylation and increased GABA release in hippocampal inhibitory interneurons were shown to cause impaired Nf1+/− mouse LTP and learning [43,48]. Consistent with this model, treating Nf1 mutant mice with a GABA<sub>A</sub> receptor antagonist (i.e., picrotoxin) restored normal LTP in the hippocampus [43]. These findings established a direct relationship between Ras regulation and GABAergic signaling relevant to the Nf1 learning deficits.
The notion that deregulated Ras activation can cause cognition dysfunction is further underscored by several human disorders caused by genetic mutations that lead to increased Ras signaling. For example, patients with Legius syndrome, a NF1-like disorder that results from inactivating mutations in the SPRED1 Ras-MEK/ MAPK regulator, exhibit mild neurocognitive deficits [49–51]. Similarly to Nf1+/− mice, defective spred1 function in mice resulted in increased MAPK signaling [49,52,53], in addition to deficits in learning and memory [54]. Costello syndrome is a Ras disorder (‘Ras-opathy’) caused by an activating mutation in the HRAS gene, leading to constitutive Ras signaling [55]. Children with Costello syndrome often have cognitive impairments, low IQs, and increased anxiety [56]. Similarly to their human counterparts, HRARasG12V knock-in mice exhibit increased anxiety behaviors and cognitive impairments [57]. Lastly, in additional models of aberrant Ras activity, pharmacological inhibition of PI3K or the MAPK kinase MEK was effective at restoring normal synaptic plasticity and learning in rodents [58–61]. Taken together, these studies demonstrate that inappropriate regulation of Ras activity can disrupt cognitive function, providing a preclinical rationale for Ras pharmacological treatments for NF1-associated cognitive deficits.

The role of cAMP in learning and memory
In Drosophila, neurofibromin modulates cAMP by activating the adenylyl cyclase 1 (AC1) homolog, rutabaga (rut) [35,36]. Although the exact mechanism remains to be elucidated, activation of rut-AC is initiated by an interaction with the C terminus of neurofibromin [39]. Further, this interaction is essential for learning and memory in flies [62,63]. Using a classic Pavlovian olfactory behavioral paradigm, flies lacking Nf1 or rut-AC show reduced performance, indicative of learning and memory impairments [62–66]. Restoration of neurofibromin expression using dNf1 heat-shock transgenes, or correction of the cAMP defect by ectopic protein kinase A (PKA) expression, restored normal learning and memory in Nf1 mutant flies [62]. Recent studies have revealed that distinct domains of Drosophila neurofibromin can modulate different components of learning and memory. Whereas mutations in the GAP domain cause defects in long-term memory, the cAMP-activating C-terminal region of neurofibromin mediates immediate memory or learning [45]. A direct link between cAMP homeostasis and cognitive defects has not been firmly established in Nf1 mouse models, but support for a role of cAMP in learning and memory derives from studies in other rodent models. In these reports, pharmacological and genetic manipulation of cAMP levels enhanced LTP, learning, memory, and hippocampal neurogenesis [67–71].

In Nf1 mutant mice, some of the defects observed in CNS neurons were found to be dependent on cAMP signaling. Following complete Nf1 loss in neural stem cells (NSCs), reduced secondary somatosensory cortex thickness was observed [72]. Further examination of these Nf1 CKO (i.e., Nf1BLBP-CKO) mice revealed that this reduction in cortical thickness resulted from decreased neuronal arborization, rather than from reductions in the total number of neurons. Using in vitro methods, it was demonstrated that neurons differentiating from Nf1-deficient NSCs also had shortened neuronal processes [72]. The biochemical basis for this defect in neuronal morphology was shown to result from impaired cAMP generation because both Nf1BLBP-CKO mouse brains and Nf1+/− primary neurons had lower cAMP levels in vitro and in vivo [72–74]. Re-expression of the Ras- regulatory NF1- GAP domain in Nf1BLBP-CKO mice did not correct the cortical thickness deficit. Similarly, expression of an activated K-Ras allele in NSCs both in vitro and in vivo did not alter neurite length. Instead, treatment of Nf1BLBP-CKO mice with pharmacological agents that increase cAMP levels restored normal cortical thickness in vivo and neurite lengths in primary Nf1+/− neurons in vitro [72–74].

Because the brains of children with NF1 are composed of neurons with reduced, not absent, NF1 gene expression, subsequent studies have employed Nf1+/− CNS neurons as a relevant model (Figure 2a). Similar to Nf1-deficient neurons, Nf1+/− CNS neurons exhibited 25% and 40% reductions in neurite (axonal) length and growth-cone area, respectively [72,73]. These abnormalities resulted from impaired cAMP generation, and were corrected by treatments that elevated intracellular cAMP levels, including Rolipram (a phosphodiesterase-4 inhibitor), non-hydrolyzable cAMP analogs, and adenylyl cyclase (AC) activators (e.g., forskolin). Conversely, blocking cAMP production, using 2,3 dioxoxygenadenosine (DDA) to inhibit adenylyl cyclase function, was sufficient to attenuate neurite extension in wild type neurons [73,74]. Lastly, neurofibromin regulation of actin cytoskeletal dynamics was found to be dependent on cAMP-mediated PKA activation of Rh/ROCK/MLC signaling [74], such that correcting the defective signaling conferred by any component along this pathway normalized Nf1+/−-axon lengths and growth cone areas in vitro (Figure 2b).

Further examination of these abnormal Nf1+/− CNS neurons also revealed small increases in programmed cell death. In studies examining the impact of reduced neurofibromin expression upon retinal ganglion cells (RGCs), optic glioma formation in Nf1 mutant strains was associated with increased RGC apoptosis [73,75]. As observed in vitro, treatment of Nf1 optic glioma-bearing mice with Rolipram (to raise intracellular cAMP levels) almost completely ameliorated the increased RGC programmed cell death in vivo [73], underscoring the importance of neurofibromin-mediated cAMP homeostasis in the maintenance of normal CNS neuronal function. Interestingly, CNS neurons with reduced Nf1 expression had similar levels of cAMP to neurons completely lacking neurofibromin expression, emphasizing the profound effect of Nf1 heterozygosity on CNS neuronal morphology and function [74]. Further studies using hippocampal neurons demonstrated a requirement of neurofibromin for dendritic spine formation, such that Nf1 knockdown reduced spine formation in mature neurons in vitro [76]. Correspondingly, dendritic spine densities were also reduced in the brains of Nf1+/− mice [77]. Together, altered dendritic spine density, axonal morphological defects, and reduced neuronal survival may lead to impaired synaptic efficacy and reduced cognitive function in children with NF1.
The role of dopamine homeostasis in learning, memory, and attention

Owing to the high prevalence of attention problems in children with NF1, stimulant medications, such as methylphenidate (MPH), have proved to be effective treatments in this affected population [2]. MPH restores dopamine homeostasis by blocking dopamine reuptake through the DA transporter (DAT), allowing increased extracellular dopamine availability in dopaminergic neurons [78,79]. Although brain dopaminergic defects have yet to be reported in children with NF1, impaired dopamine homeostasis has been shown in a Nf1 genetically engineered mouse strain [80]. In these studies, Nf1+/− mice with total Nf1 loss in GFAP+ astroglial cells (Nf1+/− −GFAP/CKO mice) were used. Similarly to Nf1−/− mice, Nf1+/− −GFAP/CKO mice had reduced exploratory behaviors and performed poorly on tests of selective and non-selective attention. Moreover, there were reduced dopamine levels and reduced postsynaptic dopamine signaling in the striatum, as measured by decreased dopamine- and cAMP-regulated phosphoprotein-32 (DARPP32) phosphorylation. In addition, cell-autonomous decreases in the growth cone areas and neurite lengths of dopaminergic neurons in vitro were observed, resulting in attenuated cell projections to the striatum in vivo. Although post-synaptic dopamine receptor expression was normal, presynaptic dopamine transporters (i.e., DAT and vesicular monoamine transporter (VMAT)) and tyrosine hydroxylase (TH) expression were significantly reduced in Nf1+/− −GFAP/CKO mice [80] (Figure 3). To provide a preclinical measure of reduced striatal dopamine levels, positron emission tomography (PET) imaging with 11C-raclopride demonstrated increased binding in the striata of Nf1+/− −GFAP/CKO mice, indicative of low endogenous dopamine [81]. Consistent with a dopamine availability deficit, and similar to children with NF1, treatment with dopamine-elevating drugs, such as MPH and L-DOPA, increased striatal dopamine levels and ameliorated attention defects in these mice [80,81].

Dopamine system function is also critical for learning and memory in rodents, such that disruption of hippocampal dopaminergic innervations and loss of hippocampal D1/D5 dopamine receptor function result in spatial learning defects [82–84]. A recent study has explored the relationship between dopamine homeostasis and learning in Nf1+/− −GFAP/CKO mice further [85]. This study found Nf1+/− −GFAP/CKO mice to have reduced dopamine levels in the hippocampus, similar to the striatum. Moreover, L-DOPA administration rescued the post-acquisition Morris watermaze probe trial deficits in vivo, and dopamine D1 receptor agonist (i.e., SKF-38393) treatment corrected the LTP abnormalities in hippocampal slice preparations in vitro. These findings, coupled with previous observations, establish defective dopaminergic function as a contributing factor important for both spatial learning/memory and attention system dysfunction in Nf1 mutant mice.

Although the precise mechanism by which neurofibromin modulates dopamine signaling has yet to be identified, these findings suggest that dopamine-targeted therapies may be useful treatments for some children with NF1-associated cognitive abnormalities. Furthermore, these studies highlight the potential utility of raclopride PET imaging to stratify children with NF1 into subgroups most likely to respond to dopamine-elevating treatments.

Figure 2. Neurofibromin regulation of neuronal morphology is cAMP-mediated. (a) Neuronal morphology (growth cone areas and neurite length) is attenuated by reduced neurofibromin expression and cAMP levels. Top panel: primary cultured Nf1−/− hippocampal neurons have decreased neurite lengths (not shown) and growth cone areas as a result of reduced cAMP generation [73]. Middle panel: stimulating adenylcyclase (AC) with forskolin (FOR) elevates cAMP levels and rescues growth cone areas in Nf1−/− hippocampal neurons. Bottom panel: decreasing cAMP levels in wild type neurons by blocking AC activity with the AC inhibitor 2,3-dideoxyadenosine (DDA) blunts growth cone areas, comparable to Nf1−/− neurons. Scale bar, 20 μm. Adapted, with permission, from [73]. (b) Schematic diagram illustrating mechanism of cAMP-mediated morphological changes. In neurons, low cAMP generation due to reduced Nf1 expression, in turn, leads to decreased PKA activation, RhoA/ROCK activity, and MLC phosphorylation. These changes contribute to impaired actin cytoskeletal dynamics, resulting in smaller growth cones and shortened axons [74]. Abbreviations: GPCR, G protein-coupled receptor; MLC, myosin light chain; PKA, protein kinase A; ROCK, Rho-associated protein kinase.
Clinical heterogeneity and implications

Although it is convenient to regard monogenetic disorders as homogenous medical conditions, the marked clinical variability between members of the same family with the same germline NF1 genetic mutation argues to the contrary. Instead, it is more likely that NF1 is composed of numerous distinct diseases, each defined by factors including patient age, patient sex, the timing of NF1 inactivation, the specific cell type, genomic modifiers, and microenvironmental influences. These factors have begun to be elucidated in cancers arising in NF1 genetically engineered mice where the timing of bi-allelic NF1 gene inactivation [86–88], the cell type (cell of origin) [89–96], the genetic (strain) background [97–99], and the non-neoplastic cell microenvironment [93,100–102] play critical deterministic roles.

Based on these observations, we propose a model of NF1-associated cognitive abnormalities in which the wide range of clinical features represents an admixture of distinct cellular and molecular etiologies. As such, there is no single molecular abnormality that underlies all of the cognitive dysfunction observed in children with NF1. Rather, the specific collection of cognitive and behavioral deficits observed in any given child with NF1 reflects the relative contributions of multiple cellular and molecular defects (Figure 4). Emerging evidence implicates specific neuronal populations, several molecular defects, and other contributing factors; as such, we envisage a model of cognitive dysfunction in which the resulting clinical phenotype reflects the interplay of all of these potential abnormalities.

First, genomic factors likely influence the specific constellation of cognitive and behavioral deficits observed in children with NF1. In this regard, attention deficits are more common in boys in the general population, but are equally represented in both sexes in children with NF1 [1]. This finding suggests that sexually dimorphic genomic factors may contribute to the phenotypes observed in prepubescent children, as have been reported for other CNS abnormalities in mice [103–105]. Second, not all CNS neuronal populations appear to be equally affected by reduced NF1 gene expression. The use of conditional knockout mouse strains has already revealed that NF1+/- forebrain pyramidal neurons do not contribute to abnormal learning and memory [48]. It is possible that distinct neuronal populations differentially respond to reduced neurofibromin expression, and contribute in unique fashions to the learning, memory, and attention deficits that characterize mice and people with a germline inactivating

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**Figure 3.** Role of dopamine in NF1-associated behavior. (a) Tyrosine hydroxylase (TH)-positive neurons in the substantia nigra project to the striatum and modulate dopamine signaling. Presynaptically, TH converts tyrosine to L-DOPA. Upon release, dopamine returns to the presynaptic dopamine pool by binding dopamine transporters or it activates adenyl cyclase (AC) by binding to postsynaptic dopamine receptors. AC activation stimulates cAMP generation and dopamine- and cAMP-regulated phosphoprotein-32 (DARPP32) phosphorylation in the striatum. (b) In NF1 mutant mice, TH expression is reduced, leading to lower striatal dopamine levels and lower effector signaling, as indicated by reduced DARPP32 phosphorylation [80]. Abnormal dopamine homeostasis also results in attention system dysfunction in the NF1 mutant model [80,81]. (c) Treatment with drugs that increase dopamine synthesis (L-DOPA), exogenous dopamine levels (dopamine), or block dopamine reuptake [e.g., methylphenidate (MPH)] restores striatal dopamine levels and reverses the attention phenotype in NF1 mutant mice [80,81]. Abbreviation: GPCR, G protein-coupled receptor.
NF1 gene mutation. Third, the specific germline NF1 gene mutation may create different abnormalities in CNS neurons that reflect the degree of neurofibromin reduction or a specific impairment in cAMP or Ras signaling. Although there are few studies examining the effect of specific NF1 gene mutations on neurofibromin expression and function, NF1*−/− mice maintained on different genetic backgrounds exhibit a wide spectrum of reduced NF1 mRNA expression [99]. Fourth, it is possible that some of the more severe neurocognitive abnormalities occasionally seen in children with NF1 may reflect total loss of neurofibromin expression in select populations of CNS neurons. Recent evidence demonstrates gross brain abnormalities following Nf1 biallelic inactivation in developing neural stem cells [106]. Further study will be required to formally evaluate this intriguing possibility. Fifth, Nf1 mutant mouse strains have been instructive in elucidating the major neurochemical defects responsible for learning, memory, and attention abnormalities associated with NF1. Intelligent use of these preclinical mouse strains may provide insights into potential treatments that reflect the predominant signaling defect (e.g., cAMP, Ras, dopamine) in individuals with NF1.

Finally, it is possible that non-neuronal cell types, including NF1*−/− oligodendrocytes, microglia, and/or astrocytes contribute to abnormal neuronal function as a result of disrupted axonal signal propagation, impaired synaptic pruning, and/or changes in glutamate or other neurotransmitter availability.
plasticity and pruning [110,111]. Increased numbers of microglia have been observed in Nf1\textsuperscript{−/−} mouse brains [100], where they represent critical modulators of optic glioma growth [101,102]. Their roles in NF1-associated learning, memory, and attention deficits have not been explored.

Concluding remarks
In this review, we have highlighted the roles of Ras, cyclic AMP, and dopamine as key molecular targets that modulate NF1-associated cognitive dysfunction. Each of these factors represents a potential therapeutic target; however, the efficacy of any particular treatment regimen will rely heavily on the specific constellation of molecular and cellular abnormalities present in a given child with NF1 (Figure 5). This idea is underscored by the outcomes of several recent NF1 clinical trials. Although treatment with the Lovastatin analog, Simvastatin, did not improve cognitive functioning in the group as a whole, it did enhance performance on some features of learning and memory in a subgroup of patients [47]. A more recent Phase I Lovastatin trial demonstrated some improvement in verbal and nonverbal memory, without any effect on attention system dysfunction [112]. Similarly, some reports have shown that MPH can improve working memory in children with ADHD [113] as well as learning in NF1 patients [2]. By considering the interplay between each of these cellular and molecular abnormalities, future approaches to managing the learning, memory, and attention system deficits in children with NF1 may consider integrating targeted therapies specific to the biochemical and neurochemical abnormalities unique to each child (Box 1).

Box 1. Outstanding questions
- How does neurofibromin regulate cAMP homeostasis in CNS neurons?
- How does neurofibromin regulate dopamine homeostasis in the brain?
- What is the relationship between neurofibromin cAMP, Ras, and dopamine regulation in CNS neurons?
- Does reduced neurofibromin function in distinct CNS neuronal populations differentially contribute to the cognitive and behavioral abnormalities observed in children with NF1?
- How do non-neuronal cells (such as microglia, oligodendrocytes, and astrocytes) influence the cognitive and behavioral abnormalities in children with NF1?
- How do combinations of molecular and cellular abnormalities result in specific cognitive profiles in children with NF1?
- What underlies the clinical heterogeneity in children with NF1-associated cognitive and behavioral abnormalities (genomic modifiers, environmental factors, patient sex, etc.)? What are the implications for treatment?
- How can we stratify children with NF1-associated cognitive and behavioral abnormalities based on their unique molecular and cellular defects? What are the implications for personalized therapy?

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