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Abnormal cerebellar development in autism spectrum disorders

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Abstract

Autism spectrum disorders (ASD) comprise a group of heterogeneous neurodevelopmental conditions characterized by impaired social interactions and repetitive behaviors with symptom onset in early infancy. The genetic risks for ASD have long been appreciated: concordance of ASD diagnosis may be as high as 90% for monozygotic twins and 30% for dizygotic twins, and hundreds of mutations in single genes have been associated with ASD. Nevertheless, only 5–30% of ASD cases can be explained by a known genetic cause, suggesting that genetics are not the only factor at play. More recently, several studies reported that up to 40% of infants with cerebellar hemorrhages and lesions are diagnosed with ASD. These hemorrhages are overrepresented in severely premature infants, who are born during a period of highly dynamic cerebellar development that encompasses an approximately five-fold size-expansion, an increase in structural complexity, and remarkable rearrangements of local neural circuits. The incidence of ASD-causing cerebellar hemorrhages during this window supports the hypothesis that abnormal cerebellar development may be a primary risk factor for ASD. However, the links between developmental deficits in the cerebellum and the neurological dysfunctions underlying ASD are not completely understood. Here, we discuss key processes in cerebellar development, what happens to the cerebellar circuit when development is interrupted, and how impaired cerebellar function leads to social and cognitive impairments. We explore a central question: is cerebellar

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development important for the generation of the social and cognitive brain or is the cerebellum part of the social and cognitive brain?

Keywords

Autism spectrum disorders; cognition; social behavior; cerebellum; connectivity; morphogenesis

Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental conditions that involve impaired social interactions and restricted or repetitive behaviors as core symptoms (DSM-5) [1]. These core symptoms must present early in the developmental period, cause impairment in social or occupational functioning, and may not be better explained by intellectual disability or social delay to meet the criteria for ASD diagnosis based on the DSM-5 [1]. The clinical presentation of symptoms and etiology for the disorder are highly diverse. There is a strong genetic component of the disease, as the concordance for ASD diagnosis in monozygotic twins is up to 90% and for dizygotic twins 30% [2]. Yet, only 5–30% of ASD cases can be explained by a mutation of a single gene or by genomic rearrangement (duplications or deletions) [3]. Interestingly, many of the monogenic, Mendelian inherited ASD genes have a high co-expression in the cerebellum. This growing list of genes includes, but is not limited to, *SHANK2* and *SHANK3*, *TSC1* and *TSC2*, and *AUTS2* [4–6].

A role for the cerebellum in ASD is further suggested by the observation that cerebellar hemorrhages or lesions early after birth increase the incidence and risk for ASD [7–10]. These cerebellar hemorrhages are especially prevalent in severely premature infants with low birthweight. As the survival of these prematurely born infants has increased significantly in the last two decades, the greater risk for ASD has been exposed and studied in more depth. Preterm birth and cerebellar hemorrhage coincide with, and may specifically disrupt, the period of highly dynamic cerebellar development that is characterized by morphologic and synaptic reorganization [10–12]. These findings have led researchers to hypothesize that there may be a developmental time window during which the cerebellum is central for the acquisition of cognitive function and social skills [13]. With an emphasis on disease susceptibility during this window, we explore potential functional links between abnormal cerebellar development and neurological deficits in ASD and highlight recent studies that underscore the importance of cerebellar connectivity and function for social and cognitive behaviors.

Anatomical evidence for a social and cognitive cerebellum

The cerebellum is easily recognizable in human by its hyper-foliated structure that allows the organized packing of ~80% of the neurons in the brain into only ~20% of its volume [14] (shown in Fig. 1A). Despite this relatively small volume, systematic unfolding of the human cerebellar surface revealed that it has nearly 80% of the total surface of the human neocortex [15]. In rodents, the cerebellum is the only foliated structure in the brain and its medial (vermis) and lateral (hemispheres) portions are roughly equal in size [16]. In higher functioning animals, though, including apes, dolphins, and seals, the cerebellar hemispheres have expanded disproportionally, raising the possibility that expansion of specific cerebellar regions may enhance functional capacity and even contribute to higher cognitive function [17]. These anatomical studies set the stage for a possible role of the cerebellum beyond motor control. Indeed, functional imaging studies in human revealed cerebellar activity in many different tasks [18–20], adding working memory, emotional and social processing, and language to core functions of the cerebellar circuit. Studies in mice show extensive, albeit indirect, connections from the cerebellum to the prefrontal cortex [21–23] (shown in Fig. 2). This suggests that the activation of cerebellar neurons during social and cognitive tasks, as observed in humans, is not merely the result of an efferent copy signal but a direct modulation of neural circuits classically associated with higher order functions such as memory, language, and social behaviors. This leads to the question, if the cerebellum is central to higher brain function, do cerebellar lesions result in social and cognitive abnormalities?

Cerebellar injury and cognitive impairments in humans

In line with evidence from cerebellar anatomy and functional imaging, adults with lesions in the cerebellum can develop cerebellar cognitive affective syndrome (CCAS), with core symptoms of impaired executive function, difficulties in spatial cognition, blunted affect or disinhibited and inappropriate behavior, and language difficulties [24–26]. Some children who have tumor resection surgery for medulloblastomas, a cerebellar tumor, also exhibit symptoms of CCAS [27], and some experience posterior fossa syndrome (PFS). PFS is characterized by a similar but often more severe constellation of symptoms including mutism, emotional lability, ataxia, hypotonia, and behavioral disturbances [28,29]. While the acute, severe presentation of PFS often resolves with time, children who experienced PFS often suffer long-term neurocognitive impairment [30–32]. The presence of linguistic, cognitive, and behavioral deficits in patients with CCAS and PFS may further implicate disruptions of cerebellar function in ASD, which is a pervasive developmental disorder characterized by an earlier onset and broader spectrum of symptom severity in these domains.

Why, then, may the cognitive and affective symptoms of ASD be more severe than CCAS? Aligned with the similarities in the clinical presentation of these entities, cerebellar abnormalities are one of the most commonly observed anatomical observations in ASD patients [33]. In the cerebellum of some ASD patients, there are findings of a reduced number of Purkinje cells [34,35], which form the computational units and sole output of the cerebellar cortex. Interestingly, this reduction occurs prenatally but after Purkinje cells migrate to their final location in the cerebellar cortex [36]. Genetic evidence also points towards a disruption of cerebellar development in ASD, as 26 monogenetic ASD genes and ASD risk genes show high co-expression in the cerebellar cortex [4], and gene expression profiles of early developing mouse [6] and human [37] Purkinje cells are enriched for ASD-associated genes. Thus, early Purkinje cell loss or aberrant transcription in Purkinje cells during developing may be key defining culprits in ASD. These early perturbations may result in more severe symptoms compared to the behavioral abnormalities observed in children and adults with cerebellar lesions (those with PFS and CCAS, respectively) because they occur during a period of highly dynamic cerebellar development. Before discussing the effects of early cerebellar injury in ASD, we will summarize the late stages of cerebellar development to highlight why genetic and mechanistic insults on the cerebellum during this period may have broad effects on cerebellar function and behavior.

Development of the cerebellar circuit

Cerebellar development follows a protracted developmental timeline compared to the cerebral cortex [11,12,28]. The first cerebellar neurons are born during mid embryogenesis, but neurogenesis and differentiation of the late-born granule cells, which are the most populous cell type in the brain, occurs during gestational week 20–40 in human and postnatally in rodents (shown in Fig. 1). Granule cell neurogenesis is promoted by the earlier-born Purkinje cells [38,39], and with the exponential expansion of the granule cell population, the cerebellum ultimately increases approximately five-fold in size and as a consequence there is a remarkable increase in the complexity of its folding patterns (shown in Fig. 1). Granule cells are also fundamental for the lamination of the cerebellar cortex as their cell bodies comprise the internal granule cell layer, and their axons project to and synapses in the molecular layer onto Purkinje cell dendrites. Sandwiched between the granule cell layer and molecular layer, is a single layer of Purkinje cell bodies. At the synaptic level, the repetitive, canonical microcircuits that makes up the cerebellar cortex also undergoes dynamic changes with the arrival of granule cells. First, granule cells make up the majority of direct synaptic input onto Purkinje cell dendrites. Second, mossy fibers, which relay motor information from the cerebral cortex to the cerebellum via the pontine nuclei and sensory information from the spinal cord via the spinal-cerebellar pathways, initially make direct contacts onto developing Purkinje cells but displace these contacts to granule cell dendrites once granule cells arrive in the internal granule cell layer. Finally, whereas each Purkinje cell is initially innervated by multiple climbing fibers, originating in the inferior olive, it is the granule cell input on the Purkinje cell dendritic tree that contributes to the pruning of supernumerary inputs until each Purkinje cell receives input from precisely one climbing fiber. Strikingly, in human these processes start before the third trimester begins and continue until up to two years after birth (detailed reviews on cerebellar development in [10–12]). Altogether, the cerebellum initiates a remarkable set of processes including dynamic changes in its size, morphology, and synaptic arrangements during the later stages of prenatal development. These processes are dependent on precisely timed and reciprocal interactions between granule cells and Purkinje cells [11,40]. Genetic or mechanic insults to either cell-population can disrupt the developmental cascade and thereby not only damage cells in which the insult occurs, but also other neurons in the cerebellar cortex, thereby amplifying the effects of the initial insult. These cellular and circuit interaction may explain why early insults in the cerebellum may lead to more profound behavioral and cognitive impairments than those found in children and adults.

Cerebellar injury and ASD in humans

As briefly introduced before, several lines of evidence set the groundwork for suggesting an important role for cerebellar dysfunction in certain etiologies of ASD: (1) the massive expansion of the cerebellum temporally coincides with the onset of pathogenesis in ASD [13]; (2) the cerebellum is prone to injury during this period of expansion [41–43]; and

(3) cerebellar injury is increasingly thought to contribute to aberrant social and linguistic functions [44–47], which are hallmarks of ASD. In this section, evidence that cerebellar injury contributes to ASD will be reviewed. The findings of cerebellar abnormalities in individuals with ASD are longstanding; earlier post-mortem studies found that individuals with ASD have a decrease in the number of Purkinje cells [48], while grossly decreased volume of the vermis has been noted [49,50]. More recently, these findings have been extended with evidence that individuals with ASD have reduced cerebellar gray matter [51,52]. In addition to findings of anatomic abnormalities in individuals with ASD, evidence of altered functional and structural connectivity between the cerebellum and the cortex, the canonical seat of neurocognitive function, has been observed in individuals with these disorders. Using Diffusion Tensor Imaging (DTI), which is an imaging modality used to evaluate myelin integrity, disruption of intrinsic cerebellar white matter tracts [53] as well as the afferent and efferent white matter tracts that convey information to and from the cerebellum [54,55], have been found in individuals with ASD. As might be expected, given these anatomic and structural abnormalities, the functional connectivity between the cerebellum and the cortex is also abnormal in individuals with ASD [56,57]. Perhaps unexpectedly, the abnormal connectivity is characterized by both increased and decreased strength of the functional connections, though intriguingly increased connectivity has been noted between the cerebellum and the sensorimotor cortex while decreased connectivity was observed between the cerebellum and areas of the cortex that are associated with cognitive function [56]. Though speculative, in this context it is interesting to note that discrepant development is often considered a hallmark of the ASD diagnosis. However, given the correlative nature of both post-mortem studies and imaging based functional and structural connectivity studies, finding a causal relationship between cerebellar injury and ASD diagnosis has been difficult.

Recently, several clinical studies have laid the groundwork for understanding how prematurity itself, as well as injury during prematurity, may affect cerebellar and cognitive development. First, Brossard-Racine et al. looked at cerebellar anatomy of pre-term (<32 weeks; PT) and term infants using magnetic resonance imaging (MRI) [58]. They found that there were abnormalities in the cerebellum of PT infants in the perinatal period including diminished cerebellar hemisphere volume and increased vermis volume compared to age equivalent babies who were imaged *in utero*. By term equivalent age, however, the hemispheric differences disappeared and only increased vermis volume persisted in the PT population compared to the term babies. Second, Herzmann et al. noted alterations in functional connectivity in both intrinsic and cerebello-cortical networks when looking at very preterm infants (<27 weeks) and term-born infants [59]. Third, looking specifically at preterm infants (<34 weeks) with cerebellar hemorrhage, Boswinkel et al. found that children with cerebellar injury indeed demonstrated altered developmental trajectories, the severity of which was associated with the extent of cerebellar hemorrhage [60]. This finding was in accordance with previous studies [61,62], further increasing the body of literature associating cerebellar injury, prematurity, and altered development. Together, these studies implicate the cerebellum as a key brain region involved in establishing the neurodevelopmental trajectory in ASD and identify late gestation as a key time period that is likely involved in this process. Outstanding questions include: What factors mediate altered cerebellar development in the preterm infants *without* parenchymal injury? Does cerebellar hemorrhage add injury to this insult, compounding the damage to an already vulnerable brain region? Can preclinical murine models provide insight into this process and provide possible neuromodulatory interventions to improve developmental trajectories?

Assessing ASD pathogenesis in murine models

There are no direct murine equivalents to hallmark behaviors included in the clinical assessment of ASD diagnosis, including abnormal social behavior and language. Nevertheless, there are several behavioral assessments in mice that can be used to assess social, cognitive, and language processing in rodents [63]. Social deficits are often assessed with the three-chamber test in which an animal is placed in three connected chambers with the middle chamber being empty and the side chambers filled with “empty vs. object”, “stranger mouse vs. object”, and “stranger mouse vs. familiar mouse” in sequential sessions [64]. Mouse models of monogenetic ASD perform poorly in differentiating the stranger mouse vs. object or familiar mouse phase of the experiments [65,66], suggesting that this test is indeed a good model for social deficits. However, this test is usually performed using adult mice, and therefore does not assess any social deficits in the neonatal period. A test for early ASD-like deficits is ultrasonic vocalizations (USV), which can be tested by maternal separation of the pups between postnatal days (P)5 to P14 and may be a good measure for mimicking the abnormal language performance that is often observed in infants with ASD. Indeed, many mouse models of monogenetic ASD show abnormalities in the frequency or duration of USVs [65,67]. Additionally, repetitive behaviors in mice are often tested by quantifying self-grooming or using the marble burying test, in which the number of marbles buried within a set time is quantified. However, such paradigms that test repetitive behaviors can be confounded by gross motor performance and have more variable outcomes in ASD models. We refer to Simmons et al. (2020) for an excellent in-depth review on the validity and limitations of behavioral tests in mouse models of ASD [68]. Next, we describe how these behavioral paradigms are affected in mouse models of monogenetic ASD, cerebellar development, and during acute cerebellar perturbations.

Evidence of cerebellar involvement in monogenetic ASD mouse models

Much of what we currently know about the cellular neuropathophysiology of ASD comes from studying monogenetic ASD mouse models. Dysfunction of cerebellar Purkinje cells and impairments in cerebellar-dependent learning, including eye-blink conditioning, have been reported in genetic mouse models of ASD ($Shank3^{+/\Delta C}$, $Mecp2^{R308/Y}$, $Cntnap2^{-/-}$, *L7-Tsc1* (*L7/Pcp2-Cre;*$Tsc1^{flox/+}$), and in the patDp (15q11–13)/+) mouse [69]. While these findings do not prove a role for the cerebellum in ASD pathogenesis, they do show that abnormal cerebellar function is prevalent and frequently observed in different ASD models. More specific evidence comes from Purkinje cell-restricted *Tcs1, Tcs2,* and *Auts2* conditional knockout mice that have impairments in social behavior and vocalizations as well as abnormal Purkinje cell function and anatomy [65,70,71]. Recent findings have now built on these initial observations by showing that Purkinje cell-specific loss of *Tcs1* results in hyperactivity of the prefrontal cortex and that modulating specific circuit nodes in the cerebellum to prefrontal cortex pathway can improve social deficits and repetitive or inflexible behaviors [72]. Taken together, abnormalities in cerebellar function are commonly observed in mouse models of monogenetic ASD and Purkinje cells-specific loss-of-function of ASD-causing genes are sufficient to drive ASD-like behavior and functional deficits in forebrain regions classically implicated in social and cognitive control. The next questions to address are whether non-genetic forms of ASD can also drive equivalent impairments, and if they do, does it occur through similar cerebellar mechanisms.

Premature birth and neonatal injury cause abnormal cerebellar function in animal models

The mechanism(s) by which disrupted cerebellar development may lead to ASD-like impairments is still relatively understudied. *ENGRAILED2,* which is heavily expressed in midbrain and hindbrain during development, is an ASD susceptibility locus [73–75] and mice lacking the transcription factor *Engrailed2* have cerebellar hypoplasia, lower Purkinje cell number, and ASD-associated behavioral impairments [76]. While this shows that genes important for cerebellar development may be associated with ASD phenotypes, it does not answer the question of how interruptions in cerebellar development mechanistically affect cerebellar function. The distinctions in the cerebellar developmental timeline with regard to birth in different mammals hinders direct modelling of premature birth in mice (shown in Fig. 1). Interestingly though, Purkinje cells in preterm baboons have structural and functional impairments [77], and a pig model of premature birth shows reduced granule cell neurogenesis [78] that can be partially rescued by providing formula supplemented with docosahexaenoic acid esterified to phosphatidylserine [79]. When we used a genetic model to impair granule cell neurogenesis in mice, we found abnormalities in the anatomical and functional maturation of Purkinje cells, as well as abnormal USVs and motor control [40]. Sathyanesan et al. (2018) modelled preterm birth and subsequent hindrance of cerebellar development by exposing postnatal mice to prolonged hypoxia, and they also found impairments in intrinsic Purkinje cell function [80].

A key observation in these various models of altered cerebellar development–whether the insult is genetic, environmental, or mechanical–is convergence of the perturbations in Purkinje cell function. It should be noted that compared to anatomical deficits, such changes in Purkinje cell function, irrespective of whether they are intrinsic and affect biophysical properties or extrinsic and affect synaptic features, are harder to assess in human ASD. This is mainly because they cannot be visualized by MRI techniques or postmortem investigation of cerebellar integrity. Nevertheless, the data showing abnormal Purkinje cell function are in line with human fMRI studies showing abnormal connectivity between the cerebellum and other brain regions in ASD subjects [59,60]. Thus, in contrast to the large and acute cerebellar lesions that characterize movement disorders, perhaps the social and cognitive deficits that characterize ASD are the result of widespread, relatively mild but cumulative developmental disruptions and abnormal Purkinje cell function. This begs the question whether specific Purkinje cell perturbations directly impair social and cognitive deficits.

Disruptions in cerebellar function lead to social and cognitive deficits in mice

Overt impairments in cerebellar function caused by disrupting synaptic vesicle release from climbing fibers onto Purkinje cells or from Purkinje cells onto the cerebellar nuclei neurons result in severe motor impairments that may conceal the presence of social deficits [81,82]. However, a myriad of recent studies used different strategies to manipulate local circuits in the cerebellum, and all these studies strongly implicate a role for cerebellar function in social, cognitive, and anxiety-like behaviors (shown in Fig. 2). For example, chemogenetic inhibition of Purkinje cells results in ASD-associated social and repetitive behaviors [83] and chemogenetic inhibition of molecular layer interneurons (which directly modulate the firing activity of Purkinje cells [84]) perturbed cognitive and social behavior in an age- and location-specific manner, while leaving locomotion and gait unaffected [85]. Specifically, chemogenetic perturbation in juveniles resulted in more profound behavioral deficits, and disrupting the function of Crus I/II led to abnormal performance in a three-chamber assay whereas disrupting the function of Lobule VII resulted in reduced grooming, a measure for repetitive behavior. Shortly after, it was found that the cerebellum drives social and reward-associated behavior through direct connections between the cerebellar nuclei and the ventral tegmental area (VTA) [86], a brain region classically appreciated as a neural reward center (shown in Fig. 2). Inhibition of this projection causes reduced social interest for a novel mouse in the three-chamber assay and activation of this projection is rewarding without an external stimulus.

Evidence of the importance of cerebellar function in complex cognitive domains is rapidly expanding. For instance, recent work shows that Purkinje-cell specific deletion of tyrosine hydroxylase impairs behavioral flexibility and reduces social interest in a three-chamber assay [87]. In addition, granule cell deletion of the $\delta GABA_A$ receptor subunit results in anxiety-like behavior as well as female-specific deficits in social behavior and maternal care [88]. It is important to note that gait and motor learning are not substantially affected in either of these manipulations. In the last few years, several studies have further underscored the importance of the cerebellum in higher order function using acute, optogenetic, manipulations of specific cell-types. Acute activation of the cerebellar cortex disrupts hippocampal function and impairs performance in a spatial memory task [89], optogenetic activation and inhibition of Purkinje cells result in a reduction or increase in aggressive behaviors, respectively [90], and activation or inhibition of cerebellar fastigial nucleus neurons projecting to the periaqueductal gray modulate fear memories [91] (shown in Fig. 2).

Despite these studies showing (in)direct connections between the cerebellum and brain regions associated with higher cognitive function, the mechanism by which the cerebellum controls cognitive behavior is still far from clear. There is some intriguing evidence that the cerebellum may control coherence between different brain regions, which may improve task performance by generating a common dynamic frame in a multi-regional brain network [92,93]. Specifically, neural activity in the cerebellum represents the coherence of neural oscillations between the hippocampus and prefrontal cortex [93], and inhibition of the cerebellar cortex disrupts this coherence and may decrease performance in a memory task [94]. Interestingly, this role of the cerebellum in modulating coherence of multiple brain regions is not restricted to cognitive function, as excitation of Purkinje cells also disrupts phase-coherence between the sensory and motor cortices during whisker stimulation [95]. Thus, while these functional studies do not confirm that disruption of cerebellar development causes early-onset cognitive, social, or language impairments directly observed in ASD, they provide compelling evidence that the cerebellum directly modulates brain-wide, neural networks involved in highly complex cognitive tasks.

Conclusion

In this review, we summarized evidence that cerebellar dysfunction occurs in monogenetic forms of ASD and that genetic and structural perturbations in the cerebellum can lead to ASD-associated symptoms. Furthermore, we introduced rapidly growing evidence showing that in healthy adults and mature mice, the cerebellum functionally modulates brain regions classically associated with cognitive and social behaviors. We propose that perturbations in cerebellar development lead to alterations in higher order function because of changes in the function of Purkinje cells, and that these neurons are integrated in a multi-node brain network that controls complex tasks including memory, language, and social interactions. This leaves a key question; what accounts for the more severe phenotypes associated with perinatal cerebellar injury compared to the sometimes subtler, yet clinically important, social and cognitive deficits seen in adults with cerebellar injury and CCAS? An intriguing hypothesis posed by the literature reviewed here may be that perinatal lesions have secondary effects on cerebellar circuit development, which then lead to more significant neurodevelopmental deficits. In this way, a relatively small perinatal lesion or perturbation during development may affect the function of a larger cerebellar region and its associated extrinsic circuitry, ultimately damaging dynamically functioning networks. Future studies could use mouse models to characterize how temporal-spatial dynamics of cerebellar cortex circuit dysfunction may lead to ASD-associated impairments. In parallel, existing mouse models can be used to uncover whether recent successes of improving motor control using deep brain stimulation targeted to the cerebellar nuclei can be extrapolated to improve cognitive and social behavior in patients with neurodevelopmental disorders [81,82,96].

In conclusion, the data summarized in this review suggest that perturbations during cerebellar development impair the intrinsic and synaptic functionality of cerebellar neurons, which in turn can lead to ASD-associated deficits because the cerebellum is an integral node in the neural network that guides complex social and cognitive behaviors. The reviewed literature demonstrates a critical role for the cerebellum in the development and maintenance of the social and cognitive brain. Moving forward, understanding precisely how the cerebellum is involved in the generation of typical social and cognitive development and why cerebellar injury alters these functions will be of central importance. We posit that the cerebellum is important for the development of the social and cognitive brain because it is part of the social and cognitive brain.

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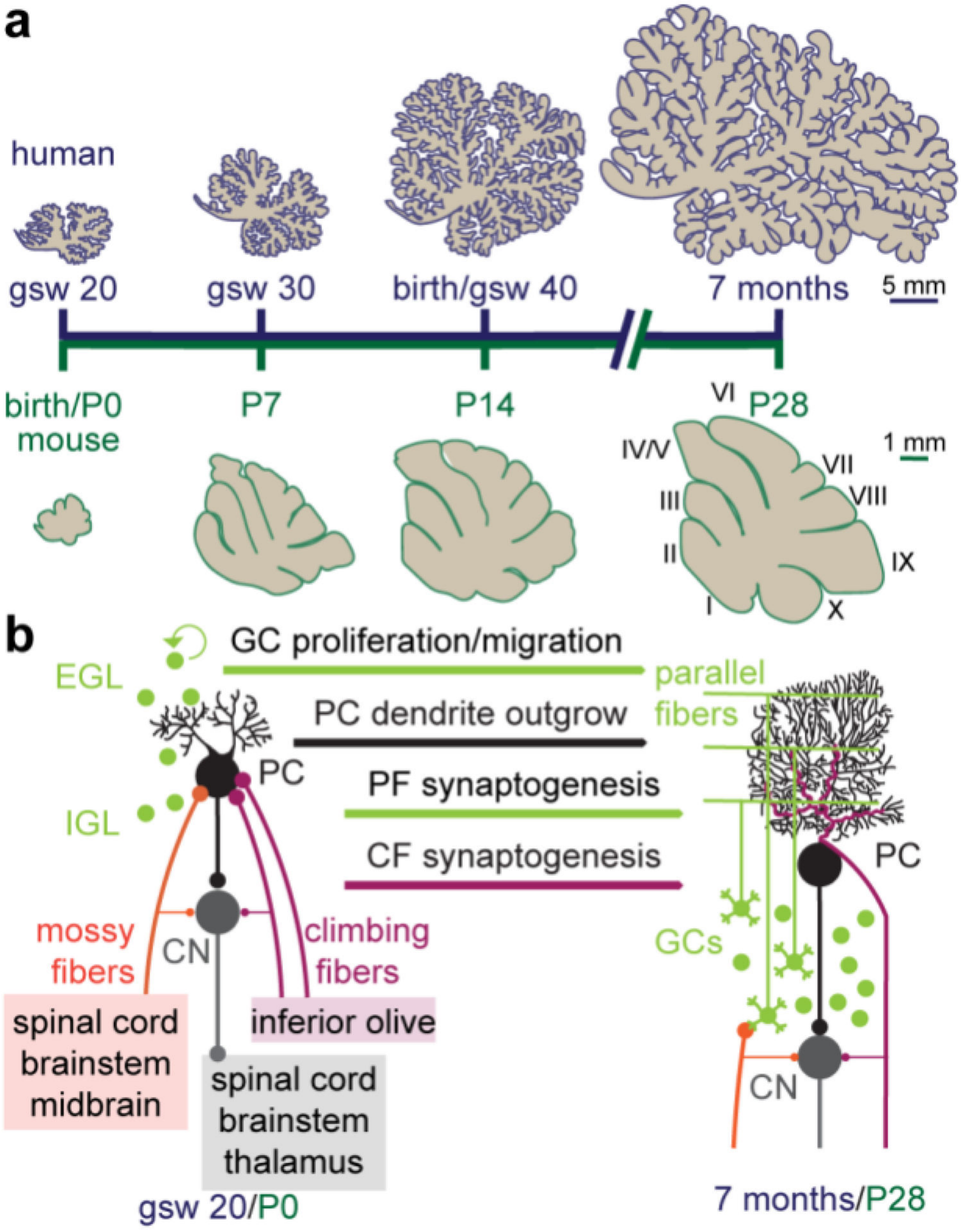

Fig. 1.

Timeline of cerebellar development in human and mouse. a. Size expansion and increased lobule complexity of the cerebellum shown from around birth. Abbreviations: gsw = gestational week; P = postnatal day. b. Changes in connectivity of the canonical cerebellar microcircuit during cerebellar development. Abbreviations: EGL= external granule cell layer; IGL = internal granule cell layer; GC = granule cell; PC = Purkinje cell; CN = cerebellar nuclei; PF = parallel fibers; CF = climbing fiber.

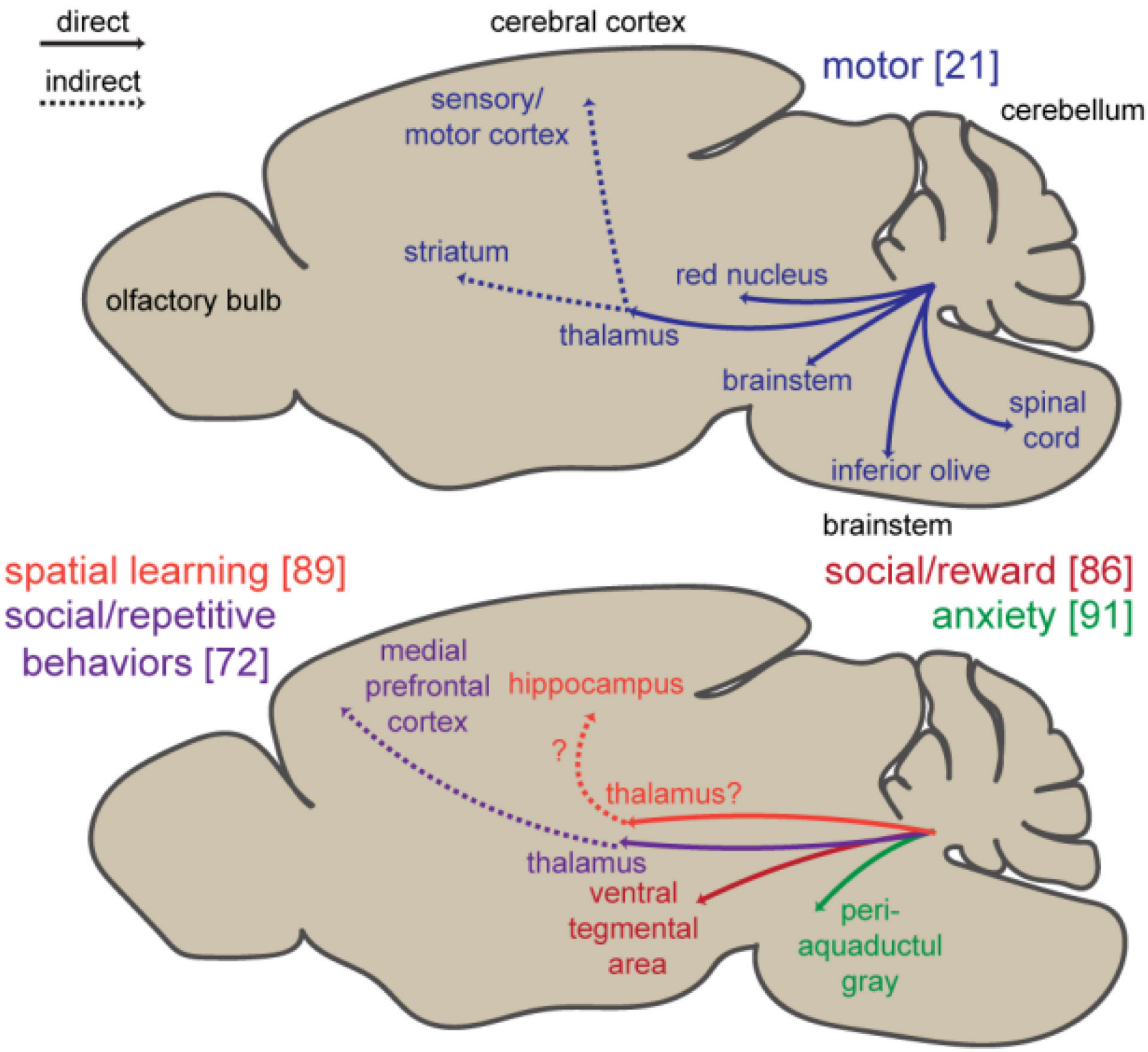

Fig. 2.

Connectivity between the cerebellar nuclei and other regions of the central nervous system. Top: cerebellar projections to brain regions classically associated with motor control, as detailed by Fujita et al, 2020 [21]. Bottom: cerebellar projections contributing to the social and cognitive behaviors, as characterized in Zeidler et al., 2020 [89]; Kelly et al., 2020 [72]; Carta et al., 2019 [86]; and Frontera et al., 2020 [91].