HHS Public Access

Author manuscript

Neurobiol Learn Mem. Author manuscript; available in PMC 2020 June 19.

Published in final edited form as:

Neurobiol Learn Mem. 2019 November ; 165: 106861. doi:10.1016/j.nlm.2018.04.016.

Cerebellar contribution to locomotor behavior: A neurodevelopmental perspective

Aaron Sathyanesan[1,*], **Vittorio Gallo**[1,2]

[1]Center for Neuroscience Research, Children's Research Institute, Children's National Health System, Washington DC, USA

[2]George Washington University School of Medicine and Health Sciences

Abstract

The developmental trajectory of the formation of cerebellar circuitry has significant implications for locomotor plasticity and adaptive learning at later stages. While there is a wealth of knowledge on the development of locomotor behavior in human infants, children, and adolescents, pre-clinical animal models have fallen behind on the study of the emergence of behavioral motifs in locomotor function across postnatal development. Since cerebellar development is protracted, it is subject to higher risk of genetic or environmental disruption, potentially leading to abnormal behavioral development. This highlights the need for more sophisticated and specific functional analyses of adaptive cerebellar behavior within the context of whole-body locomotion across the entire span of postnatal development. Here we review evidence on cerebellar contribution to adaptive locomotor behavior, highlighting methodologies employed to quantify and categorize behavior at different developmental stages, with the ultimate goal of following the course of early behavioral alterations in neurodevelopmental disorders. Since experimental paradigms used to study cerebellar behavior are lacking in both specificity and applicability to locomotor contexts, we highlight the use of the Erasmus Ladder – an advanced, computerized, fully automated system to quantify adaptive cerebellar learning in conjunction with locomotor function. Finally, we emphasize the need to develop objective, quantitative, behavioral tasks which can track changes in developmental trajectories rather than endpoint measurement at the adult stage of behavior.

Keywords

cerebellum; cerebellar learning; locomotor behavior; adaptive learning; neurodevelopment; Erasmus Ladder

Introduction

In his widely influential work, *The Study of Instinct*, Nikolaas Tinbergen defined behavior as "The total movements made by the intact animal" (Tinbergen, 1951). While simplistic in its formulation, this definition highlights the ethological relevance of motor behavior as a "final form" of how organisms interact with their environment. The cerebellum is critical to

*Corresponding author; asathyanesan@childrensnational.org.

the control of motor behavior, especially within the context of whole-body locomotion, which we define here as coordinated movement involving the whole-body (including the head, back, and extremities) along a defined spatial trajectory. However, locomotor behavior - and adaptive motor skills in general - do not suddenly emerge as fully developed skills. In humans, adaptive locomotor behavior evolves over the course of postnatal development (Berger and Adolph, 2007). Analogously, rodents - which make up the bulk of pre-clinical animal model studies - also display postnatal development in locomotor behavior.

Most studies involving animal models of neurodevelopmental disorders do not track the development of motor behavior at different postnatal stages, but instead use adult behavior as an endpoint measurement. The conclusions drawn from such analyses of behavior run the risk of offering coarse explanations of mechanism which may not be translationally relevant to developmental human studies. Generally speaking, the fact that clinicians do not wait till the infant or child reaches the adult stage to intervene therapeutically and measure behavioral outcomes should prompt neuroscientists to rethink how behavior is typically analyzed in pre-clinical animal models of neurodevelopmental disorders.

However, the tendency to treat behavioral analysis as something that is only measured at the adult stage is not without cause. There are conceptual and design challenges in measuring cerebellar and locomotor behavior at younger stages of development. Some of these challenges are linked to the development of specific circuitry within the cerebellum, as well as its afferent and efferent pathways (White and Sillitoe, 2013). Other challenges have more to do with how “specific” a given task is to cerebellar function. It must be noted at the outset, however, that locomotion is not exclusively a cerebellum-dependent task. Locomotion is a complex behavior, enabled by the recruitment of different neural circuits, both central and peripheral. Many excellent reviews have been written on the aspects of locomotion which highlight distinct aspects of motor control modulated by spinal circuits (Catela, Shin, and Dasen, 2015; Kiehn, 2016; Rybak, Dougherty, and Shevtsova, 2015), brainstem (Ryczko and Dubuc, 2017), basal ganglia (Grillner and Robertson, 2015; 2016; Yin, 2016), and cortical circuits (Drew and Marigold, 2015). In this review, we will address cerebellar contributions to locomotion, with a special focus on the development of locomotor plasticity and adaptive cerebellar learning. We will finally highlight technological advances in measuring adaptive cerebellar function, as well as provide a conceptual framework for the future development of neurobehavioral tasks that can be applied across different stages of postnatal development.

Box: Importance of measuring behavior as a function of development

Almost a decade following publication of *The Study of Instinct*, Tinbergen described his famous “four questions” for organizing the analysis of animal behavior: ‘survival value’ or function, ‘evolution’, ‘causation’ or mechanism, and ‘ontogeny’ (Tinbergen, 1963). The fourth question of ‘ontogeny’ or development was added following pointed criticism of Tinbergen’s work by biologists such as Daniel S. Lehrman who wrote an incisive critique of Tinbergen’s, as well as his collaborator Konrad Lorenz’s framework of behavior (Lehrman, 1953). In the 28 page critique published in the year 1953 in the *Quarterly Review of Biology*, Lehrman drew on then-available data on rat maternal

behavior, and pecking behavior in the chick, to identify weaknesses in the Tinbergen and Lorenz binary distinction of “innate” versus “not-innate” behaviors. Lehrman pointed out that in these examples of developmentally regulated behavior:

> “…analysis of the developmental process involved shows that the behavior patterns concerned are not unitary, autonomously developing things, but rather that they emerge ontogenetically in complex ways from the previously developed organization of the organism in a given setting.”

Krakauer et al. (2017) in a philosophical paper addressing the need for more comprehensive analysis of behavior in neuroscience studies advocate for a more pluralistic approach to studying behavior, drawing on David Marr’s three organizational levels of understanding the brain: computation (level 1), algorithm (level 2), and implementation (level 3). They point out correctly that following technological advances in manipulating neural function, neuroscience studies in recent years have become biased to favor an implementation-based explanation of behavior at the expense of computational or algorithmic approaches. We generally agree with this conclusion. However, while Krakauer *et al* incorporate Tinbergen’s four questions into their pluralistic model, and mention development in passing while discussing the development of vocal production in marmoset monkeys, they do not explicitly describe the importance of ‘ontogeny’ or development of behavior. Taking a cue from the Krakauer pluralistic model, we propose that studying the processes by which the algorithms for a particular behavior develop over the course of postnatal development are critical to understanding how the algorithms may become ‘buggy’ in cases such as adult psychiatric disease. In the context of neurodevelopment, this is akin to the initial algorithm being ‘debugged’ over various pre-final or ‘beta’ versions of the adult algorithm. Alternatively, there could be multiple ‘algorithm’ overhauls with different ‘computations’ and ‘implementation’ over the course of postnatal brain development.

Development of the wiring diagram of the cerebellar cortex

Development of locomotor behavior is inextricably linked to formation and maturation of specific neural circuits in the CNS. Adaptive locomotor learning and plasticity are a core functional feature of cerebellar cortical circuitry, and form an important subset within the repertoire of locomotor behavioral motifs. In this review, we define adaptive learning as a form of associative conditioned learning involving locomotor adaptation in response to a goal-disruptive perturbation. The parts of the cerebellar wiring diagram which are necessary for adaptive learning include 1. Excitatory climbing fiber (CF) input to Purkinje cells (PCs) from the inferior olive (IO) 2. Excitatory parallel fiber (PF) input to PCs from granule cells (GCs) receiving input from mossy fibers (MF) of pre-cerebellar nuclei, 3. Inhibitory input to PCs from molecular layer interneurons (MLIs), comprising stellate cells and basket cells, and 4. Inhibitory input to cerebellar nuclei (CN) from PCs, which form the final output of the cerebellar cortex.

In humans and mice, the major cell types required for establishing the wiring diagram (PCs and first wave of GCs, as well as GABAergic interneurons) are born *in utero*. Cerebellar

circuitry itself begins to form concurrent with dynamic developmental processes occurring during the interface of the second and third trimester of gestation in humans (Volpe, 2009), and in the first two postnatal weeks in mice (Hashimoto, Ichikawa, Kitamura, Watanabe, and Kano, 2009; Watt, Cuntz, Mori, Nusser, Sjostrom, and Hausser, 2009; van Welie, Smith, and Watt, 2011). The proliferative and migratory phase of cells in the external granule layer (EGL) is linked to the formation of PFs from GCs as they migrate to their future location in the internal granule layer (IGL). PFs thus begin to establish contact with PC dendritic arbors during the migratory phase as GCs pass through the molecular layer. After 40 weeks of gestation, the EGL in the human cerebellum is considerably reduced in size and gradually disappears around the 11^{th} postnatal month (Abraham, Tornoczky, Kosztolanyi, and Seress, 2001). While PC dendritic arbor formation and synaptogenesis begins around mid-gestation in humans, in mice much of the dendritic arborization and synaptogenesis occurs during postnatal development. Finally, in both rodents and humans, PCs display axonal targeting to cerebellar nuclei at birth (Rakic and Sidman, 1970; White and Sillitoe, 2013), but important events such as synaptic pruning and refinement continue till ~P30 in mouse, and likely up to the end of the first postnatal year in humans (Zecevic and Rakic, 1976).

Developmental hallmarks of adaptive locomotor behavior

Two aspects of locomotor behavior are important to address in terms of development: one involves lower-level basal features such as gait and posture, and the second aspect involves higher-level task-based features, such as associative learning and challenge-based realtime motor adaptation. As an example for lower-level features, consider a mouse pup ‘learning’ to move in its home-nest, or a human infant ‘learning’ to crawl on a level surface. Higher-level features would involve negative geotropism displayed by mouse neonates or a juvenile mouse pup learning to escape a predator by jumping or transitioning from exploratory low-velocity locomotor state to a high-velocity ‘flight’ state as soon as its olfactory system detects the predator odor. For humans, comparative tasks could involve an infant learning how to navigate a flight of stairs, and an adolescent learning howto tackle an opponent while dribbling a soccer ball (Figure 1). Lower-level features are not necessarily a direct functional outcome of cerebellar circuitry, but they nevertheless affect task-related higher-level features. Consequently, cerebellar damage during development may cause changes in lower-level features in addition to higher-level features. While lower-level locomotor features can be assessed at all ages, higher-level features are dependent on the development of related brain regions. Example, if the animal’s adaptive response to an unconditioned stimulus is being measured, then the conditioned response to a conditioning stimulus depends on which stimuli is being presented and which brain region is responsible for the conditioned response (visual system for paired light stimulation, or auditory system fora high-pitched conditioning tone).

In human infants, locomotion is not a simplistic hard-wired response, but is reflective of a repertoire of learned mechanisms with a large degree of plasticity (Adolph and Franchak, 2017). Similar to free-running mice on a running wheel, healthy infants take an average of ~2400 steps per hour committing up to 17 falls per hour, travelling in total the length of approximately 8 U.S. football fields, while learning howto walk (Adolph, Cole, Komati, Garciaguirre, Badaly, Lingeman, Chan, and Sotsky, 2012). Importantly, once infants begin

walking, fall rates reduce. This indicates some form of adaptive learning through practice and experience across different trials. It is not possible to conclude if this is a cerebellar-mediated strategy, although the cerebellar cortex is no doubt involved in practice-based, trial-by-trial adaptive locomotor learning (Heiney, Wohl, Chettih, Ruffolo, and Medina, 2014; ten Brinke, Boele, Spanke, Potters, Kornysheva, Wulff, AC, Koekkoek, and De Zeeuw, 2015). A more direct answer would involve some form of conditioned learning paradigm wherein an obstacle in the path of locomotion is paired to a conditioning stimulus like a sound stimulus. In a challenging environment, when infants are presented with an obstacle such as an illusory “drop-off”, infants learn through exploration how best to navigate the obstacle. This clearly involves cortical circuitry for planning movements (Spampinato and Celnik, 2017; Svoboda and Li, 2017), since it is operating on longer timescales as opposed to cerebellar adaptive learning which occurs on sub-second timescales. Nevertheless, emerging evidence from mouse studies indicates that the cerebellum is also involved in the feedforward control of voluntary movement through cerebello-thalamic-cortical loops (Pisotta and Molinari, 2014). Whichever may be the case, the fact that infants can integrate vestibular and visual pathways to make judgements on how best to navigate obstacles in the path of locomotion indicates that future experiments can more carefully test the role of adaptive cerebellar learning in these situations. One possible avenue of future research involves the use of immersive virtual reality goggles or platforms using the Xbox Kinect to study locomotor adaptation tasks (Synofzik and Ilg, 2014).

Cerebellar contribution to lower level locomotor features: Lessons from injury and pathology

One of the behavioral hallmarks of early cerebellar injury or childhood onset genetic ataxia is abnormal gait and postural control (Fogel, 2012). Children affected with diseases such as Friedreich’s Ataxia and Ataxia Telangiectasia display gait deficits due to inter- and intra-limb miscoordination, highlighting the importance of cerebellar circuitry to gait control during development. This is because cerebellar nuclei receive information via sensory afferents from visual and vestibular regions. Lesion studies in cats showed that the fastigial and vestibular nuclei receive spinocerebellar afferents conveying peripheral sensory information, and vestibular input, respectively (Morton and Bastian, 2004; Takakusaki, 2017). These localized lesion studies typically involved unilateral surgical removal or coagulation of the cerebellar cortex or nuclei, followed by kinematic analysis. The affected cerebellar nuclei thus potentially serve as a multisensory integration site in the cerebellum. Consequently, injury to the developing cerebellum, such as in prematurity-related causes, potentially causes structural abnormalities to sensorimotor and other afferents (Limperopoulos, 2010).

Apart from receiving afferents, the cerebellum also exerts control over cortical regions via cerebello-thalamic-cortical loops (Watson, Becker, Apps, and Jones, 2014), The interpositus, which is present in the intermediate zone of the cerebellar hemispheres sends out projections to the cortex thus affecting walking patterns by modulating motor cortical networks (Morton and Bastian, 2004). The fastigial nuclei (FN) and the parieto-insular vestibular cortex (PIVC) together serve to potentially register body movement information (Shaikh, Ghasia,

Dickman, and Angelaki, 2005). While this information from the PIVC and FN may be required for anticipatory postural control, a study by Timmann & Horak (2001) (Timmann and Horak, 2001) found that adult patients with a cerebellar-related disease or injury did not display many abnormalities in postural control. The lateral zone of the cerebellum receives input from the primary motor, ad premotor regions among others, and sends out projections via the dentate nucleus to diverse cortical regions. Finally, the flocculus and paraflocculus are critical for eye-related movements. PCs in the flocculus control the vestibulo-ocular reflex as well as balance control which is a determinant of optimal gait characteristics (Ito, 1998).

Cerebellar contribution to higher level locomotor features

Human patients with cerebellar disease quite often show gait ataxia, however, since the cerebellar cortex specializes in error-based conditional, adaptive learning, it is imperative to address the role of adaptive learning within a locomotor context, and how this is affected in cases of injury. Surprisingly, the number of studies addressing cerebellar locomotor adaptation is quite small compared to the number of studies employing arm-reaching or eye movement tasks. Although relatively few in number, the handful of human studies available convincingly demonstrate the critical nature of cerebellar function in mediating locomotor adaptive learning (Hoogkamer, Bruijn, Sunaert, Swinnen, Van Calenbergh, and Duysens, 2015; Morton and Bastian, 2006).

In an early set of experiments on cats traversing a horizontal ladder, Andersson & Armstrong (1987) showed that PCs fired complex spikes when an unexpected perturbation (descent of a ladder rung by 2 cm) was placed in the path of locomotion. These experiments showed that locomotor adaptation via the cerebellar cortex has similar functional modes as other task-based cerebellar learning paradigms such as the vestibulo-ocular reflex (VOR) or the tone-eyeblink-airpuff conditioning paradigm. It is well known that in task-based paradigms as VOR and eyeblink conditioning - following a particular number of learning trials - PCs generate a conditioned response which tracks the conditioning stimulus, before the unconditioned stimulus is presented (Ohyama, Nores, Murphy, and Mauk, 2003). Functional MRI (fMRI) studies demonstrate that, within the context of eyeblink conditioning, this mechanism of CR generation via PC complex spikes may be at play in the human cerebellum as well (Gerwig, Kolb, and Timmann, 2007).

The split-belt training paradigm is an adaptive cerebellar task wherein the subject walks on a treadmill, with two independent tracks for each leg. These independent (split) treadmills can then be controlled to have different speeds, forcing gait adaptation. Studies have shown this task to be cerebellar-dependent (Morton & Bastian, 2006). Using this task, Morton & Bastian (2006) demonstrated that cerebellar patients are capable of reactive, feedback-based locomotor control, similar to healthy controls. However, unlike healthy controls, cerebellar patients cannot adapt their movement to predictive changes. In another study, transcranial magnetic stimulation (TMS) was used to assess the relative contributions of cerebellum and M1 primary motor cortex (Jayaram, Galea, Bastian, and Celnik, 2011). The authors of this study used TMS to induce cerebellar brain inhibition (CBI), reckoning that if PCs employ long-term depression (LTD) as a mechanism for adaptive learning, then TMS-driven CBI

would be reduced following locomotor adaptation. This is exactly what the authors observed. However, TMS induction of M1 resulted in excitability in a task-non-specific manner. These studies suggest strongly that similar circuitry is recruited in locomotor adaptive tasks mediated via the cerebellar cortex as in other classical conditioning tasks.

In a split-belt study involving children, Vasudevan et al (2011) demonstrated the striking finding that children as old as 11 years are not capable of adapting to the splitbelt treadmill paradigm as well as adults (Vasudevan, Torres-Oviedo, Morton, Yang, and Bastian, 2011). In fact, children performed as badly as adult cerebellar patients. This suggests that although 'mature' gait may be found in children approximately 7 years of age (Adolph and Franchak, 2017), the circuitry for adaptive locomotor function may still be immature till much later in childhood.

While human studies are just beginning to explore the adaptive mechanisms at play in the cerebellum in the context of whole-body locomotion, rodent studies are woefully lacking. To date, to our knowledge, no systematic study has been performed on younger stages of mouse adaptive locomotor development. Since childhood movement disorders may progress to much worse forms in adulthood, it is important to study preclinical models of mouse locomotor development in a systematic and comprehensive manner. Important recent work has begun to address the neurodevelopmental dimension of cerebellar function in mouse models using conventional tests such as horizontal bars, hindpaw clasping, rotarod, and stationary beam (Jacquelin, Strazielle, and Lalonde, 2012; Lalonde and Strazielle, 2015).

The Erasmus Ladder: An integrated tool to study lower-level and higher-level locomotor features in pre-clinical animal models

Most animal studies which claim a cerebellar mediated behavior paradigm use non-specific behavioral tests. Some tasks only address lower level locomotor features (such as a gait analysis system) or are too prone to extra-cerebellar contributions in the readout (accelerating rotarod) (Mann and Chesselet, 2015). The Erasmus Ladder is a platform which seeks to address the gaps in the behavioral paradigms currently used by researchers studying adaptive locomotor cerebellar function (Vinueza Veloz, Zhou, Bosman, Potters, Negrello, Seepers, Strydis, Koekkoek, and De Zeeuw, 2014) by integrating both lower-level and higher-level measurements. The Erasmus Ladder (Figure 2 – schematic, Figure 3 - actual) is an advanced, fully automated computerized behavioral system which can track both locomotor performance as well as adaptive cerebellar learning.

Basic design:

The Erasmus Ladder is a fully automated behavioral setup comprising a horizontal ladder with pressure-sensitive rungs (Figure 3). Each of the rungs is connected to a central integrating processor. When a mouse steps on a rung, the pressure on the rung activates a sensor that logs each step into the processor. As the mouse walks along the entire horizontal ladder, rungs that have been stepped are registered into the processor, thus the stepping patterns of each mouse are generated. The Erasmus ladder software has a standard protocol to test cerebellar behavior, consisting of four training sessions followed by four challenge

sessions, with one session performed per day. Each training session (sessions 1-4; Figure 2B) consists of 42 “unperturbed” trials where no obstacle challenge is presented. Each training trial consists of a light cue followed by an air cue after which the mouse leaves the goal-box and traverses the ladder to the opposite goal-box. The “resting state interval” setpoint or the time the mouse spends in the goal-box between trials is set to 15 seconds with an interval randomization of ± 5 seconds, meaning that prior to any given trial, the resting state interval randomly ranges from 10 to 20 seconds. Once the animal leaves the goal-box and is on the ladder, a light automatic tail-wind is applied to motivate the animal to reach the opposite goal-box. During challenge sessions (sessions 5-8; Figure 2B), the 42 trials are presented as randomly sequenced categories of “unconditioned stimulus only” (US-only), “conditioning stimulus only” (CS-only), or “paired”. For the US-only trials, animals confront a computer-controlled obstacle (US; Figure 2A) – which consists of a randomly activated obstacle to block the path of movement at some point along the ladder. For CS-only trials, a high-pitch warning tone is randomly presented while the animal is at some point along the ladder. And for paired trials, CS is presented followed by US with an inter-stimulus interval (ISI) of 250 milliseconds (Figure 2B). In addition to the standard protocols, the Erasmus Ladder software v1.1 allows for user-designed customized protocols to further analyze locomotor behavior. For example, an extended version of the standard protocol can be created where instead of four challenge sessions, the number of challenge sessions can be increased to eight to test for differences in latency of locomotor learning.

Analysis of Behavioral Metrics

The Erasmus Ladder system is capable of generating a wide range of metrics to analyze locomotor coordination and adaptive cerebellar learning. In particular, the Erasmus Ladder software can be used to measure the following metrics:

1. Missteps: the average percentage of steps categorized as non-optimal movement onto the lower misstep rungs (figure 2A, dark blue rungs; 2B, grey rungs) as opposed to the default walking rungs (figure 2A, light blue rungs;2B, light orange rungs). Missteps are a measurement of locomotor performance which is dependent on whole-body locomotor coordination (figure 4 a).

2. Backsteps: the average percentage of steps categorized as backward movement on the ladder during a particular trial. This can be used as an internal control metric, which is a measure of motivation to complete the Erasmus Ladder task (figure 4 b).

3. Jumps: the average percentage of steps categorized as movement which results in a mouse making steps crossing more than two rungs on the same side (figure 4 c). This metric can be used as a measure of anxiety or hypersensitivity while performing the task. If jump percentage is very high (>10%), then this mouse can be rejected from data analysis as an outlier.

4. Short steps and long steps: the percentage of steps categorized as movement to an adjacent rung on the same side (Figure 2A, “Short step”) or the rung just following the adjacent rung on the same side (Figure 2A, “Long step”). These metrics which are categorized as “steptypes” are an indicator of basal locomotor strategy in traversing the

Ladder, as well as a readout of dynamic locomotor coordination in an adaptive cerebellar task (figure 4 d).

5. Pre-perturbation steptime: millisecond precision time-difference (Δt) between rung activations just ahead of the obstacle (Figure 2A, "Δt = Pre-perturbation") on the same side.

6. Post-perturbation steptime: millisecond precision time-difference (Δt) between activated rung just before the obstacle (Figure 2A, "Δt = Post-perturbation") and the rung just after the obstacle on the same side. Obstacles are automatically raised in challenge sessions, randomly – a single obstacle is presented per trial. Obstacle presentation consists computer prediction of obstacle position based on mouse movement kinetics, and automated physical presentation of the obstacle. Comparative analysis of pre- and post-perturbation steptimes informs the presence or absence of locomotor adaptation in response to the unconditioned stimulus (US; random obstacle) (figure 4 e) or 'adaptive fluidity of motion' which is disrupted in mouse models of cerebellar dysfunction as well as human ataxias (Manto, Bower, Conforto, Delgado-Garcia, da Guarda, Gerwig, Habas, Hagura, Ivry, Marien, Molinari, Naito, Nowak, Oulad Ben Taib, Pelisson, Tesche, Tilikete, and Timmann, 2012).

Advantages of using the Erasmus Ladder:

As mentioned previously, the biggest advantage of using the ladder is the integration of measuring locomotor performance such as gait dynamics and coordination, as well as an associative, adaptive, conditioned learning paradigm to study cerebellar function. For every experiment, individual session quantification can be obtained and processed. Compared to traditional beam-walk assays, the presence of motivating cues (air and light) to enter the ladder path, and a ramped tail wind to complete the ladder trials is quite helpful since this maintains consistency and increases the number of animals used in the experiments without much experimenter intervention. In addition to cerebellar function, non-cerebellar function can also be measured by monitoring the behavior inside the goalbox. This can be easily used to analyze potential confounding variables.

Drawbacks of using the Erasmus Ladder:

While the use of a horizontal ladder setup is advantageous to study basal locomotor coordination deficits within the first few sessions, the distance between consecutive rungs is a limiting factor as to the age at which a mouse can be tested. In our hands, we have obtained reliable readouts of cerebellar behavior using the ladder at P23. However, it is not possible to measure cerebellar behavior below this age for the C57/BL6 strain since the smaller body size of the mice prevents a 'natural' stepping pattern on the current inter-rung distance (distance between adjacent rungs). A modular design wherein an intermediate set of rungs for much younger mice can be attached to the current version of the ladder would be a huge advantage, permitting the analysis of cerebellar behavior at much younger stages. Additional problems include data analysis which does not permit the analysis of raw data – rather the data is processed through a proprietary analysis package. This would be limiting for more high resolution data such as rung to rung changes in steptime in say an optogenetic stimulation paradigm.

Other limiting factors regarding the Erasmus Ladder

While the Erasmus Ladder is a robust tool for cerebellar behavioral research in a locomotor context, not many studies have used the Ladder. We think that there could be multiple reasons for this. First, the Erasmus Ladder is a relatively recent innovation compared to other more standard paradigms. Second, setting up and optimizing the Ladder requires dedicated and focused effort by researchers, in addition to a well-organized neurobehavior core facility. Third, cost may be a factor in preventing the Ladder to be more widely used. However, granted that this is an indispensable tool for cerebellar research, we refer interested researchers to Noldus Inc. for information regarding current costs.

Future Directions

Future applications in development using the Erasmus Ladder include optic fiber tethered experiments which can be performed as the animal is traversing the ladder. Using this set-up, we would effectively be able to measure and manipulate cerebellar function in a cell type-specific manner. Ca^{2+} fiber photometry (Gunaydin, Grosenick, Finkelstein, Kauvar, Fenno, Adhikari, Lammel, Mirzabekov, Airan, Zalocusky, Tye, Anikeeva, Malenka, and Deisseroth, 2014) enabled by genetically encoded Ca^{2+} indicators such as GCaMP6s and 6f would enable realtime measurement of cellular activity using cell-specific *Cre* recombinase driver lines (Figure 5 a) such as *Pcp2-Cre* (for PCs), *nNos-CreER* (for molecular layer interneurons), or *Gabra6-cre* (for granule cells). Using realtime Ca^{2+} activity data, while the animal is performing adaptive locomotor tasks on the Ladder will enable correlation with population-level neuronal activity. This will be especially insightful when measuring neuronal responses during the adaptive learning paradigm, since a correlation between neuronal activity and cerebellar learning has not been systematically analyzed for a locomotor context. Further, using optogenetics (using a similar Cre-based strategy mentioned earlier), we will be able to bridge the gap between deep locomotor behavioral analysis as well as circuit perturbations on a millisecond timescale. This would allow for modular design of experiments wherein we can study different levels of locomotor behavior on the Ladder. As an example, to test the hypothesis that pre-trial PC firing alters task performance, behavioral alterations can be measured while PCs are optogenetically stimulated or inhibited in the goalboxes compared to when they are stimulated when the trial is in progress (Figure 5 b). Since the most recent version of the Erasmus Ladder software does not have a module for the integration of triggering an external device such as an LED driver for optogenetics experiments, an independent platform such as Bonsai (Lopes, Bonacchi, Frazao, Neto, Atallah, Soares, Moreira, Matias, Itskov, Correia, Medina, Calcaterra, Dreosti, Paton, and Kampff, 2015) can be used as a suitable workaround to accomplish ROI-based triggering as described in Figure 5 b. Further, since basal deficits in ataxic mouse lines which exhibit reduced PC firing (such as in many SCA models), we can test the hypothesis that optogenetic amelioration of PC firing results in an improvement in basal locomotor function in ataxia models with reduced firing. This can also then be tested for the adaptive learning paradigm. On a very basic level, this will permit identification of cerebellar neuronal subtypes which are critical for locomotor behavior and adaptive learning. More advanced experiments, which could potentially include the simultaneous measurement of neuronal activity using optrode array recordings will address questions of

systems-level contributions of cerebellar circuitry to locomotor function and how changes in circuitry due to neurological diseases may manifest in abnormal locomotion.

Considerations for development of novel adaptive locomotor tasks

Currently, there is a knowledge divide between biological studies of animal locomotion and cerebellar function. Locomotor studies typically focus on spinal circuitry, whereas cerebellar studies in animal models generally use restrictive and ethologically less-relevant behaviors such as eyeblink conditioning. Some groups are working to bridge this gap using advanced data-driven analysis of locomotion within a cerebellar context (Machado, Darmohray, Fayad, Marques, and Carey, 2015; Vinueza Veloz et al., 2014). To better understand how cerebellar function contributes to locomotor behavior, there is much to draw from spinal circuitry research. One prominent example in mice is the circuit-modular characterization of gaits such as walk, trot, and bound, using kinematic analysis (Akay, 2014; Akay, Tourtellotte, Arber, and Jessell, 2014; Bellardita and Kiehn, 2015; Kiehn, 2016). Future studies focused on cerebellar function could potentially analyze how the modularity of these gaits changes over postnatal development and how these gaits may change when cerebellar development is altered due to neonatal injury or genetic factors. High speed kinematic analysis using paradigms such as the LocoMouse (Machado et al., 2015) may aid in characterizing these developmental changes. However, since adaptive learning is not captured by the LocoMouse, alternative methods may have to be developed.

In designing an adaptive cerebellar locomotor task for a neurodevelopmental context, two factors would have to be considered. The first is the nature of the conditioning stimulus. For this, development of sensory systems would have to be paid close attention to. For example, in mice, ear opening occurs at P3, and early reports speculated that auditory-cue conditioning could possibly be performed as early as P4 (Williams and Scott, 1954). It must be emphasized, however, that postnatal inner ear functional development is completed only at P10-P12 (Bulankina and Moser, 2012). Similarly, canonical visual system development is also not completed at this age (Shen and Colonnese, 2016). Although some reports indicate that auditory conditioning can be performed in rats as young as P12 (Hyson and Rudy, 1984; Rudy and Hyson, 1982), in terms of a cerebellar context, reliable auditory conditioning can only be performed at P17-P18 onward (Goldsberry and Freeman, 2017; Nicholson and Freeman, 2003). Thus, age must be strictly controlled while designing control experiments for possible adaptive tasks. To design cerebellar tasks at much earlier ages, utilizing the olfactory system may be a potential avenue, considering its greater importance at neonatal stages compared to other sensory systems. Indeed, olfactory conditioning using an odorant CS has been used successfully in neonatal rats as young as P2 and mice as young as P0-P3 (Logan, Brunet, Webb, Cutforth, Ngai, and Stowers, 2012; Alleva and Calamandrei, 1986; Armstrong, DeVito, and Cleland, 2006; Bouslama, Durand, Chauviere, Van den Bergh, and Gallego, 2005; Rudy and Cheatle, 1977). The second factor to consider is the unconditioned stimulus (US). For this purpose, certain behaviors or reflexes which occur early in development can be used to potentially design adaptive cerebellar tasks. The choice of US must be carefully considered since the relationship between CS and US must obey the temporal constraints of cerebellar function. A wide range of US may be useful to leverage at early ages. Some examples include apetitive/nutritive stimuli (such as a 10% sucrose

solution, or milk infusion), tactile stimulation (such as stroking), or an aversive stimulus (such as salt solution). While these US types may be generally useful for adaptive tasks, within a motor context these may be relatively harder to incorporate. Sokoloff et al (2015) have used spontaneous myoclonic twitches during active sleep in neonatal rats as a readout to correlate complex and simple spike activity in Purkinje cells (Sokoloff, Plumeau, Mukherjee, and Blumberg, 2015). This is a useful and readily accessible behavior to assess cerebellar cortical function during development, however, a localized reflex such as a twitch may not encompass our definition of coordinated whole-body movement. More in line with the goals outlined in this review, neonatal behaviors such as the righting reflex (rodent neonates will right their body position when they fall on their backs), or negative geotropism (neonates will turn up against the direction of an inclined plane) (Fox, 1965), have high potential for use in a conditioned adaptive task. Additionally, recent evidence suggests that a particular kind of photoavoidance behavior may emerge much earlier in development. Melanopsin-dependent avoidance to light in the blue region elicits a robust “turning” behavior in neonates as young as P6 (Johnson, Wu, Donovan, Majumdar, Renteria, Porco, Van Gelder, and Copenhagen, 2010). The presence of these postnatal motor reflexes indicate that some circuits may be active early in development and available to use as a tool for conceptualizing an unconditioned stimulus. Further exploration of the ontogeny of such early behavior will be important to design novel adaptive cerebellar paradigms in neonatal and juvenile animals.

Conclusion

Cerebellar behavior has been well studied in the context of single limb tasks in both humans and animals. However, to study locomotor adaptation, more careful and rigorous experimental analysis should be applied. Additionally, the development of behavioral changes must be paid attention to in order to better understand how the underlying changes in circuitry pertain to actual ethological relevance. While many tools exist to study cerebellar behavior, most of these tools are non-specific and too simplistic. The Erasmus Ladder offers an automated and comprehensive solution to studying cerebellar behavior in both juvenile and adult stages, integrating both basal as well as adaptive modes of function. Finally, we emphasize the need to explore early behaviors and reflexes which can be utilized to study adaptive cerebellar function within a locomotor task framework.

MATERIALS AND METHODS

Normal adult C57BL/6 mice of both sexes were used for behavioral experiments. All animals were handled in accordance to the Institutional Animal Care and Use Committee (IACUC) of Children’s National Medical Center and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

ACKNOWLEDGEMENTS

This work is supported by DC-IDDRC grant U54-HD090257 awarded to VG. We thank Reinko Roelofs (Noldus Inc.) for technical discussions. We thank Dr. Chris de Zeeuw (Erasmus MC, Rotterdam) for critical assessment of our project design. We thank Dr. Joshua Corbin (Director) and Dr. Li Wang (Manager) of the Animal Neurobehavior Core at the Children’s Research Institute, Children’s National Health System. We also thank Angelin Devanand, OTR/L for helpful discussions regarding development of human locomotor behavior.

Abbreviations:

PC	Purkinje cell
MLI	molecular layer interneuron
GC	granule cell
EGL	external granule layer
IGL	internal granule layer
CF	climbing fiber
PF	parallel fiber
MF	mossy fiber
IO	inferior olive
CN	cerebellar nuclei
FN	fastigial nuclei
PIVC	parieto-insular vestibular cortex
CS	conditioning stimulus
US	unconditioned stimulus
ISI	inter-stimulus interval

REFERENCES

Abraham H, Tornoczky T, Kosztolanyi G, & Seress L (2001). Cell formation in the cortical layers of the developing human cerebellum. Int J Dev Neurosci, 19, 53–62. [PubMed: 11226755]

Adolph KE, Cole WG, Komati M, Garciaguirre JS, Badaly D, Lingeman JM, Chan GL, & Sotsky RB (2012). How do you learn to walk? Thousands of steps and dozens of falls per day. Psychol Sci, 23, 1387–1394. [PubMed: 23085640]

Adolph KE, & Franchak JM (2017). The development of motor behavior. Wiley Interdiscip Rev Cogn Sci, 8.

Akay T (2014). Long-term measurement of muscle denervation and locomotor behavior in individual wild-type and ALS model mice. J Neurophysiol, 111, 694–703. [PubMed: 24174653]

Akay T, Tourtellotte WG, Arber S, & Jessell TM (2014). Degradation of mouse locomotor pattern in the absence of proprioceptive sensory feedback. Proc Natl Acad Sci U S A, 111, 16877–16882. [PubMed: 25389309]

Alleva E, & Calamandrei G (1986). Odor-aversion learning and retention span in neonatal mouse pups. Behav Neural Biol, 46, 348–357. [PubMed: 3028365]

Andersson G, & Armstrong DM (1987). Complex spikes in Purkinje cells in the lateral vermis (b zone) of the cat cerebellum during locomotion. J Physiol, 385, 107–134. [PubMed: 3656160]

Armstrong CM, DeVito LM, & Cleland TA (2006). One-trial associative odor learning in neonatal mice. Chem Senses, 31, 343–349. [PubMed: 16495436]

Bellardita C, & Kiehn O (2015). Phenotypic characterization of speed-associated gait changes in mice reveals modular organization of locomotor networks. Curr Biol, 25, 1426–1436. [PubMed: 25959968]

Berger SE, & Adolph KE (2007). Learning and development in infant locomotion. Prog Brain Res, 164, 237–255. [PubMed: 17920435]

Bouslama M, Durand E, Chauviere L, Van den Bergh O, & Gallego J (2005). Olfactory classical conditioning in newborn mice. Behav Brain Res, 161, 102–106. [PubMed: 15904716]

Bulankina AV, & Moser T (2012). Neural circuit development in the mammalian cochlea. Physiology (Bethesda), 27, 100–112. [PubMed: 22505666]

Catela C, Shin MM, & Dasen JS (2015). Assembly and function of spinal circuits for motor control. Annu Rev Cell Dev Biol, 31, 669–698. [PubMed: 26393773]

Drew T, & Marigold DS (2015). Taking the next step: cortical contributions to the control of locomotion. Curr Opin Neurobiol, 33, 25–33. [PubMed: 25643847]

Fogel BL (2012). Childhood cerebellar ataxia. J Child Neurol, 27, 1138–1145. [PubMed: 22764177]

Fox WM (1965). Reflex-ontogeny and behavioural development of the mouse. Animal Behaviour, 13, 234–IN235. [PubMed: 5835840]

Gerwig M, Kolb FP, & Timmann D (2007). The involvement of the human cerebellum in eyeblink conditioning. Cerebellum, 6, 38–57. [PubMed: 17366265]

Goldsberry ME, & Freeman JH (2017). Sensory system development influences the ontogeny of trace eyeblink conditioning. Dev Psychobiol, 59, 70–76. [PubMed: 27540891]

Grillner S, & Robertson B (2015). The basal ganglia downstream control of brainstem motor centres--an evolutionarily conserved strategy. Curr Opin Neurobiol, 33, 47–52. [PubMed: 25682058]

Grillner S, & Robertson B (2016). The Basal Ganglia Over 500 Million Years. Curr Biol, 26, R1088–R1100. [PubMed: 27780050]

Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV, Fenno LE, Adhikari A, Lammel S, Mirzabekov JJ, Airan RD, Zalocusky KA, Tye KM, Anikeeva P, Malenka RC, & Deisseroth K (2014). Natural neural projection dynamics underlying social behavior. Cell, 157, 1535–1551. [PubMed: 24949967]

Hashimoto K, Ichikawa R, Kitamura K, Watanabe M, & Kano M (2009). Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum. Neuron, 63, 106–118. [PubMed: 19607796]

Heiney SA, Wohl MP, Chettih SN, Ruffolo LI, & Medina JF (2014). Cerebellar-dependent expression of motor learning during eyeblink conditioning in head-fixed mice. J Neurosci, 34, 14845–14853. [PubMed: 25378152]

Hoogkamer W, Bruijn SM, Sunaert S, Swinnen SP, Van Calenbergh F, & Duysens J (2015). Adaptation and aftereffects of split-belt walking in cerebellar lesion patients. J Neurophysiol, 114, 1693–1704. [PubMed: 26203113]

Hyson RL, & Rudy JW (1984). Ontogenesis of learning: II. Variation in the rat's reflexive and learned responses to acoustic stimulation. Dev Psychobiol, 17, 263–283. [PubMed: 6724143]

Ito M (1998). Cerebellar learning in the vestibulo-ocular reflex. Trends Cogn Sci, 2, 313–321. [PubMed: 21227227]

Jacquelin C, Strazielle C, & Lalonde R (2012). Neurologic function during developmental and adult stages in Dab1(scm) (scrambler) mutant mice. Behav Brain Res, 226, 265–273. [PubMed: 21945093]

Jayaram G, Galea JM, Bastian AJ, & Celnik P (2011). Human locomotor adaptive learning is proportional to depression of cerebellar excitability. Cereb Cortex, 21, 1901–1909. [PubMed: 21239392]

Johnson J, Wu V, Donovan M, Majumdar S, Renteria RC, Porco T, Van Gelder RN, & Copenhagen DR (2010). Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A, 107, 17374–17378. [PubMed: 20855606]

Kiehn O (2016). Decoding the organization of spinal circuits that control locomotion. Nat Rev Neurosci, 17, 224–238. [PubMed: 26935168]

Krakauer JW, Ghazanfar AA, Gomez-Marin A, MacIver MA, & Poeppel D (2017). Neuroscience Needs Behavior: Correcting a Reductionist Bias. Neuron, 93, 480–490. [PubMed: 28182904]

Lalonde R, & Strazielle C (2015). Behavioral effects of neonatal lesions on the cerebellar system. Int J Dev Neurosci, 43, 58–65. [PubMed: 25907855]

Lehrman DS (1953). A critique of Konrad Lorenz's theory of instinctive behavior. Q Rev Biol, 28, 337–363. [PubMed: 13121237]

Lerner TN, Shilyansky C, Davidson TJ, Evans KE, Beier KT, Zalocusky KA, Crow AK, Malenka RC, Luo L, Tomer R, & Deisseroth K (2015). Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. Cell, 162, 635–647. [PubMed: 26232229]

Limperopoulos C (2010). Extreme prematurity, cerebellar injury, and autism. Semin Pediatr Neurol, 17, 25–29. [PubMed: 20434689]

Logan DW, Brunet LJ, Webb WR, Cutforth T, Ngai J, & Stowers L (2012). Learned recognition of maternal signature odors mediates the first suckling episode in mice. Curr Biol, 22, 1998–2007. [PubMed: 23041191]

Lopes G, Bonacchi N, Frazao J, Neto JP, Atallah BV, Soares S, Moreira L, Matias S, Itskov PM, Correia PA, Medina RE, Calcaterra L, Dreosti E, Paton JJ, & Kampff AR (2015). Bonsai: an event-based framework for processing and controlling data streams. Front Neuroinform, 9, 7. [PubMed: 25904861]

Machado AS, Darmohray DM, Fayad J, Marques HG, & Carey MR (2015). A quantitative framework for whole-body coordination reveals specific deficits in freely walking ataxic mice. Elife, 4.

Mann A, & Chesselet M-F (2015). Chapter 8 - Techniques for Motor Assessment in Rodents A2 - LeDoux, Mark S, Movement Disorders (Second Edition) (pp. 139–157). Boston: Academic Press.

Manto M, Bower JM, Conforto AB, Delgado-Garcia JM, da Guarda SN, Gerwig M, Habas C, Hagura N, Ivry RB, Marien P, Molinari M, Naito E, Nowak DA, Oulad Ben Taib N, Pelisson D, Tesche CD, Tilikete C, & Timmann D (2012). Consensus paper: roles of the cerebellum in motor control--the diversity of ideas on cerebellar involvement in movement. Cerebellum, 11, 457–487. [PubMed: 22161499]

Morton SM, & Bastian AJ (2004). Cerebellar control of balance and locomotion. Neuroscientist, 10, 247–259. [PubMed: 15155063]

Morton SM, & Bastian AJ (2006). Cerebellar contributions to locomotor adaptations during splitbelt treadmill walking. J Neurosci, 26, 9107–9116. [PubMed: 16957067]

Nicholson DA, & Freeman JH Jr. (2003). Addition of inhibition in the olivocerebellar system and the ontogeny of a motor memory. Nat Neurosci, 6, 532–537. [PubMed: 12692555]

Ohyama T, Nores WL, Murphy M, & Mauk MD (2003). What the cerebellum computes. Trends Neurosci, 26, 222–227. [PubMed: 12689774]

Pisotta I, & Molinari M (2014). Cerebellar contribution to feedforward control of locomotion. Front Hum Neurosci, 8, 475. [PubMed: 25009490]

Rakic P, & Sidman RL (1970). Histogenesis of cortical layers in human cerebellum, particularly the lamina dissecans. J Comp Neurol, 139, 473–500. [PubMed: 4195699]

Rudy J, & Cheatle M (1977). Odor-aversion learning in neonatal rats. Science, 198, 845–846. [PubMed: 918668]

Rudy JW, & Hyson RL (1982). Consummatory response conditioning to an auditory stimulus in neonatal rats. Behav Neural Biol, 34, 209–214. [PubMed: 7082264]

Rybak IA, Dougherty KJ, & Shevtsova NA (2015). Organization of the Mammalian Locomotor CPG: Review of Computational Model and Circuit Architectures Based on Genetically Identified Spinal Interneurons(1,2,3). eNeuro, 2.

Ryczko D, & Dubuc R (2017). Dopamine and the Brainstem Locomotor Networks: From Lamprey to Human. Front Neurosci, 11, 295. [PubMed: 28603482]

Shaikh AG, Ghasia FF, Dickman JD, & Angelaki DE (2005). Properties of cerebellar fastigial neurons during translation, rotation, and eye movements. J Neurophysiol, 93, 853–863. [PubMed: 15371498]

Shen J, & Colonnese MT (2016). Development of Activity in the Mouse Visual Cortex. J Neurosci, 36, 12259–12275. [PubMed: 27903733]

Sokoloff G, Plumeau AM, Mukherjee D, & Blumberg MS (2015). Twitch-related and rhythmic activation of the developing cerebellar cortex. J Neurophysiol, 114, 1746–1756. [PubMed: 26156383]

Spampinato D, & Celnik P (2017). Temporal dynamics of cerebellar and motor cortex physiological processes during motor skill learning. Sci Rep, 7, 40715. [PubMed: 28091578]

Svoboda K, & Li N (2017). Neural mechanisms of movement planning: motor cortex and beyond. Curr Opin Neurobiol, 49, 33–41. [PubMed: 29172091]

Synofzik M, & Ilg W (2014). Motor training in degenerative spinocerebellar disease: ataxia-specific improvements by intensive physiotherapy and exergames. Biomed Res Int, 2014, 583507. [PubMed: 24877117]

Takakusaki K (2017). Functional Neuroanatomy for Posture and Gait Control. J Mov Disord, 10, 1–17. [PubMed: 28122432]

ten Brinke MM, Boele HJ, Spanke JK, Potters JW, Kornysheva K, Wulff P, AC IJ, Koekkoek SK, & De Zeeuw CI (2015). Evolving Models of Pavlovian Conditioning: Cerebellar Cortical Dynamics in Awake Behaving Mice. Cell Rep, 13, 1977–1988. [PubMed: 26655909]

Timmann D, & Horak FB (2001). Perturbed step initiation in cerebellar subjects: 2. Modification of anticipatory postural adjustments. Exp Brain Res, 141, 110–120. [PubMed: 11685415]

Tinbergen N (1951). The study of instinct. Oxford Eng.: Clarendon Press.

Tinbergen N (1963). On aims and methods of Ethology. Zeitschrift für Tierpsychologie, 20, 410–433.

van Welie I, Smith IT, & Watt AJ (2011). The metamorphosis of the developing cerebellar microcircuit. Curr Opin Neurobiol, 21, 245–253. [PubMed: 21353528]

Vasudevan EV, Torres-Oviedo G, Morton SM, Yang JF, & Bastian AJ (2011). Younger is not always better: development of locomotor adaptation from childhood to adulthood. J Neurosci, 31, 3055–3065. [PubMed: 21414926]

Vinueza Veloz MF, Zhou K, Bosman LW, Potters JW, Negrello M, Seepers RM, Strydis C, Koekkoek SK, & De Zeeuw CI (2014). Cerebellar control of gait and interlimb coordination. Brain Struct Funct.

Volpe JJ (2009). Cerebellum of the premature infant: rapidly developing, vulnerable, clinically important. J Child Neurol, 24, 1085–1104. [PubMed: 19745085]

Watson TC, Becker N, Apps R, & Jones MW (2014). Back to front: cerebellar connections and interactions with the prefrontal cortex. Front Syst Neurosci, 8, 4. [PubMed: 24550789]

Watt AJ, Cuntz H, Mori M, Nusser Z, Sjostrom PJ, & Hausser M (2009). Traveling waves in developing cerebellar cortex mediated by asymmetrical Purkinje cell connectivity. Nat Neurosci, 12, 463–473. [PubMed: 19287389]

White JJ, & Sillitoe RV (2013). Development of the cerebellum: from gene expression patterns to circuit maps. Wiley Interdiscip Rev Dev Biol, 2, 149–164. [PubMed: 23799634]

Williams E, & Scott JP (1954). The Development of Social Behavior Patterns in the Mouse, in Relation To Natural Periods 1). Behaviour, 6, 35–64.

Yin HH (2016). The Basal Ganglia in Action. Neuroscientist.

Zecevic N, & Rakic P (1976). Differentiation of Purkinje cells and their relationship to other components of developing cerebellar cortex in man. J Comp Neurol, 167, 27–47. [PubMed: 818132]

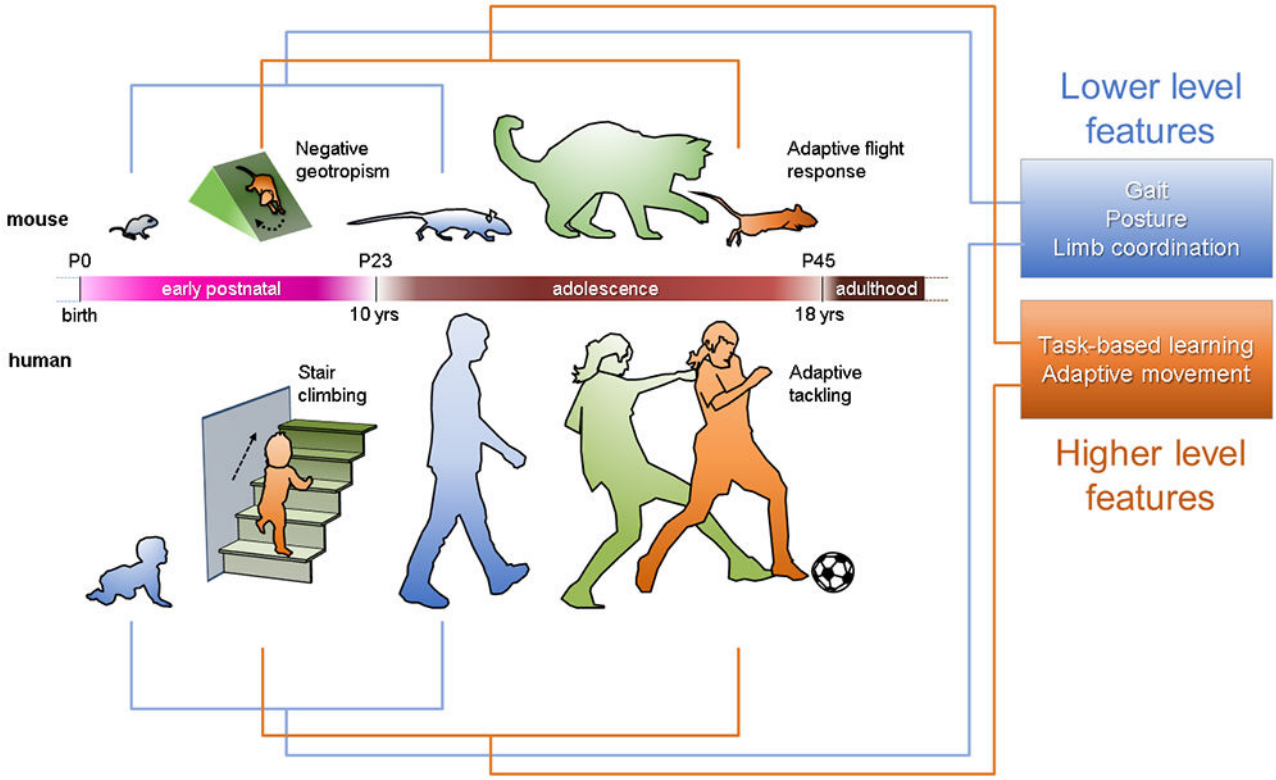

Figure 1. Levels of locomotor behavior in humans and mice across development.

Lower level features (blue) represent basal locomotor function such as gait, posture, and coordination of limbs over the course of development (timeline). Higher level features include task-based learning where movement is coordinated to adapt to external rules or constraints such as an infant learning how to climb stairs in which case whole body coordination is adapted to a sequential stepping pattern, or a mouse neonate displaying negative geotropism. In adolescent stages, comparative tasks could involve the skill of dribbling a soccer ball while avoiding an opponent or adaptive tackling. In the case of mice, an example of higher order tasks could involve escaping a vicinity by jumping, following an associated predatory olfactory cue from a cat. In this schematic, low-level features and subjects are depicted by the color blue. For higher level tasks, subjects performing tasks are depicted by the color tan, and the perturbation is depicted by the color green.

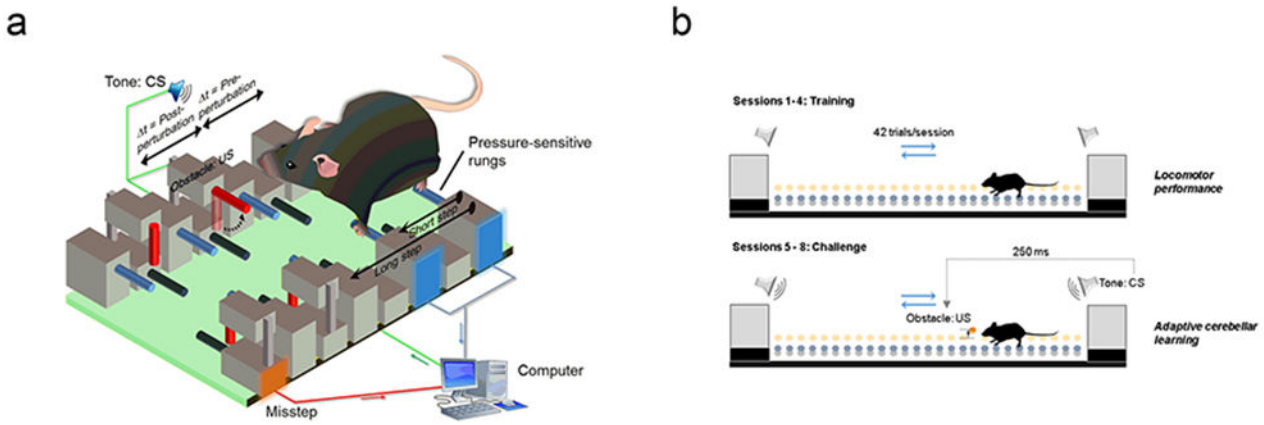

Figure 2. Cerebellar behavioral analysis using the Erasmus Ladder.

(a) A cartoon snapshot (not drawn to scale) showing mouse locomotor behavior on a small portion of the Erasmus Ladder. Step dynamics are captured using pressure sensitive rungs (blue). Stepping patterns are categorized into “short steps” or “long steps”, as well as backsteps, jumps, (not shown). Locomotor performance is assessed by percentage of “missteps” (navy blue lower rung, marked orange). Adaptive cerebellar learning is quantified using an associative conditioning paradigm involving a high-pitch tone (conditioning stimulus, CS) followed by the spatially-random activation of an obstacle (red, unconditioned stimulus, US) along the path of movement. (b) Side view cartoon of the Erasmus ladder showing the default walking rungs (light blue), misstep rungs (dark grey), obstacles (light orange), goal boxes (light grey), speakers (silver grey), and a test mouse (black). A typical Erasmus Ladder experiment consists of eight sessions, with 42 trials per session. A single trial involves the mouse moving from one goal box (grey) to the other. The first four sessions (1-4) primarily measure locomotor performance (top), while the last four sessions (5-8) primarily test adaptive cerebellar function (bottom). Interstimulus interval between CS (tone) and US (activation of obstacle, orange) is 250 ms.

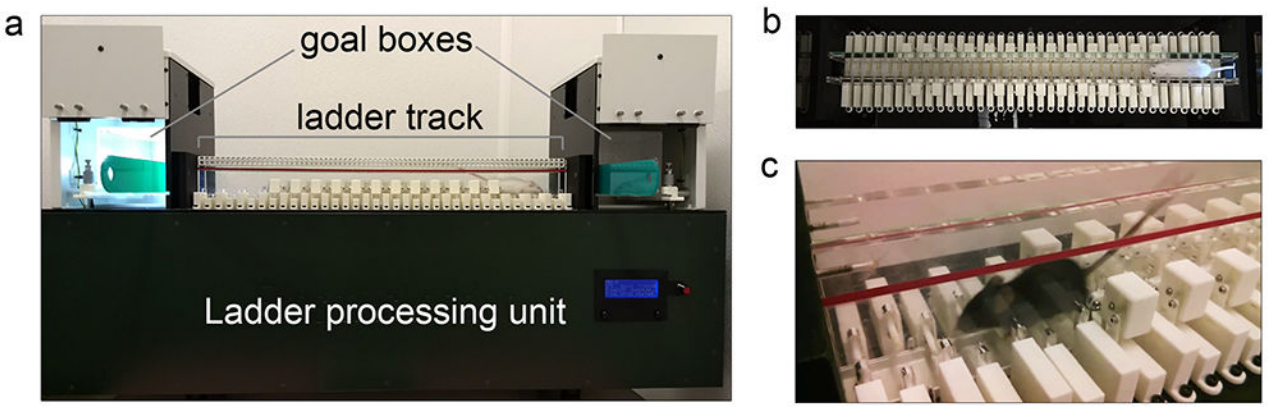

Figure 3. Erasmus Ladder.

Different views of an actual Erasmus Ladder with labeled parts (a) Side view showing an adult BALB/c mouse moving from the left goal box (light cue activated) to the right goal box. (b) Top view of the ladder (c) close-up image of a juvenile C57/BL6 mouse (P25) traversing the ladder.

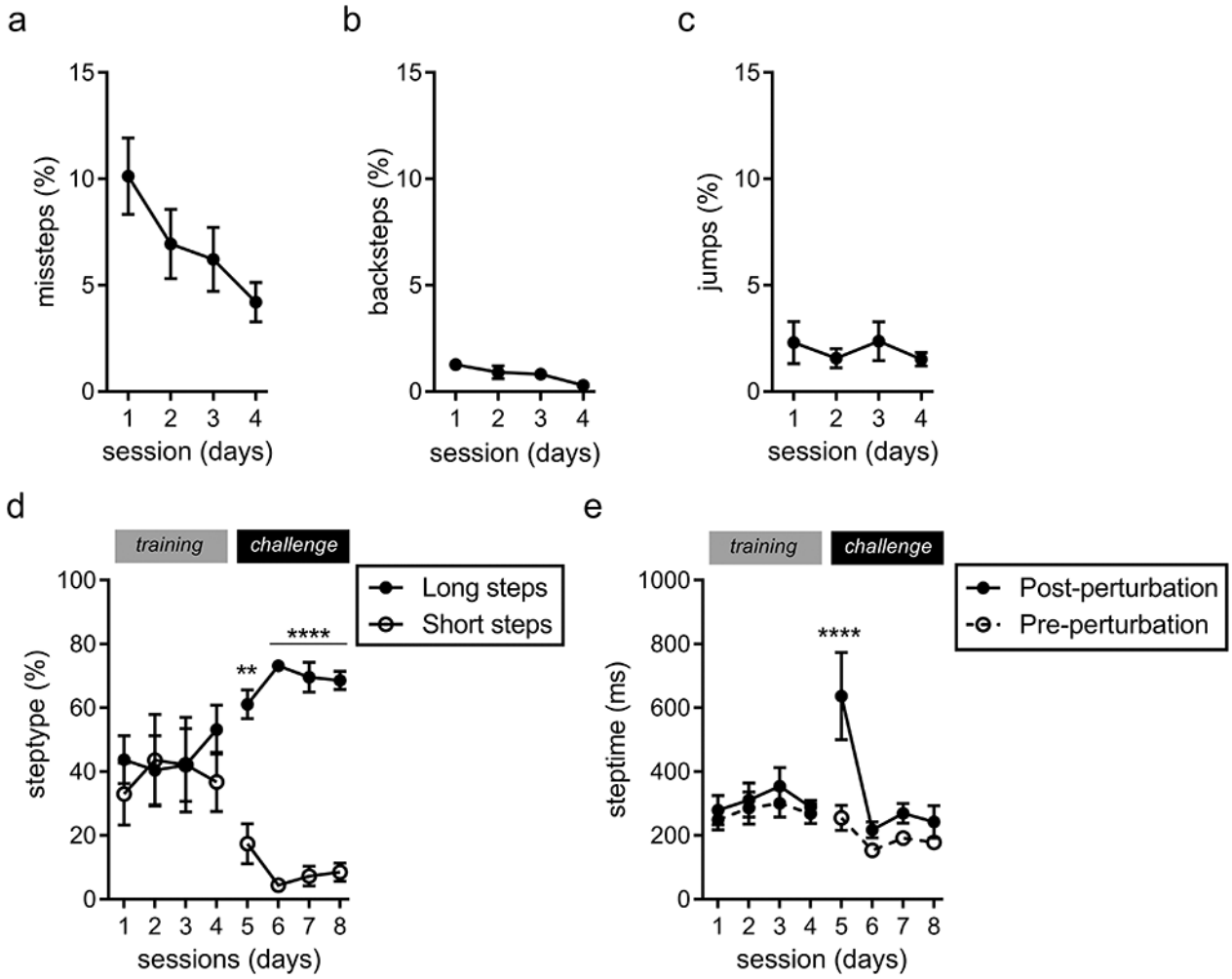

Figure 4. Quantifiable metrics using Erasmus Ladder.

Data obtained using the Erasmus Ladder can be used to quantify different locomotor behaviors. Here, data is shown for normal P60 adult C57 BL/6 mice (a) Locomotor performance can be measured using missteps across different sessions. (b) Bacskteps are recorded when an animal steps in the reverse direction to the goal box. This is an indicator of an animal's motivation to complete the Ladder task. (c) Jumps are recorded when an animal crosses more than two rungs on the same side while behaving on the ladder. This is an indicator of an animal's anxiety or state of sensitivity during the task. (d) Short steps are recorded when an animal steps on the subsequent rung on the same side; long steps are recorded when animal steps on the next-adjacent to the subsequent rung on the same side (see figure 2). For adult animals, while there is no significant difference between percentage of short and long steps during the training session, there are statistically significant differences in steptype during the challenge sessions. (e) Steptime measurements can be obtained to compare step dynamics during the training and challenge sessions. See text for detailed explanation of parameters. On session 5, there is a statistically significant difference between pre- and post-perturbation steptimes indicating incomplete association of the CS and US. By session 6, there is no difference, indicating the presence of adaptive cerebellar

learning. For panels d and e, n = 5 animals, except for session 5 in e, where n = 4. Ordinary Two-way ANOVA ($\alpha = 0.05$) was used in d and e, followed by Sidak's multiple comparison within a session. For panel d, steptypes - Interaction: F (7, 64) = 6.497 ($P < 0.0001$), Row factor (session): F (7, 64) = 0.1763 ($P = 0.9892$), Colum factor (Steptype): F (1, 64) = 61.91 ($P < 0.0001$); Sidak's multiple comparison – sessions 1-4: $P > 0.05$, session 5: $P = 0.003$, session 6-8: $P < 0.0001$. For panel e, steptimes – Interaction: F (7, 62) = 3.359 ($P = 0.0042$), Row factor (session): F (7, 62) = 6.254 ($P < 0.0001$), Column factor (steptime): F (1, 62) = 15.02 ($P = 0.0003$); Sidak's multiple comparison – sessions 1-4 and 9-11: $P > 0.05$, session 5: $P < 0.0001$.

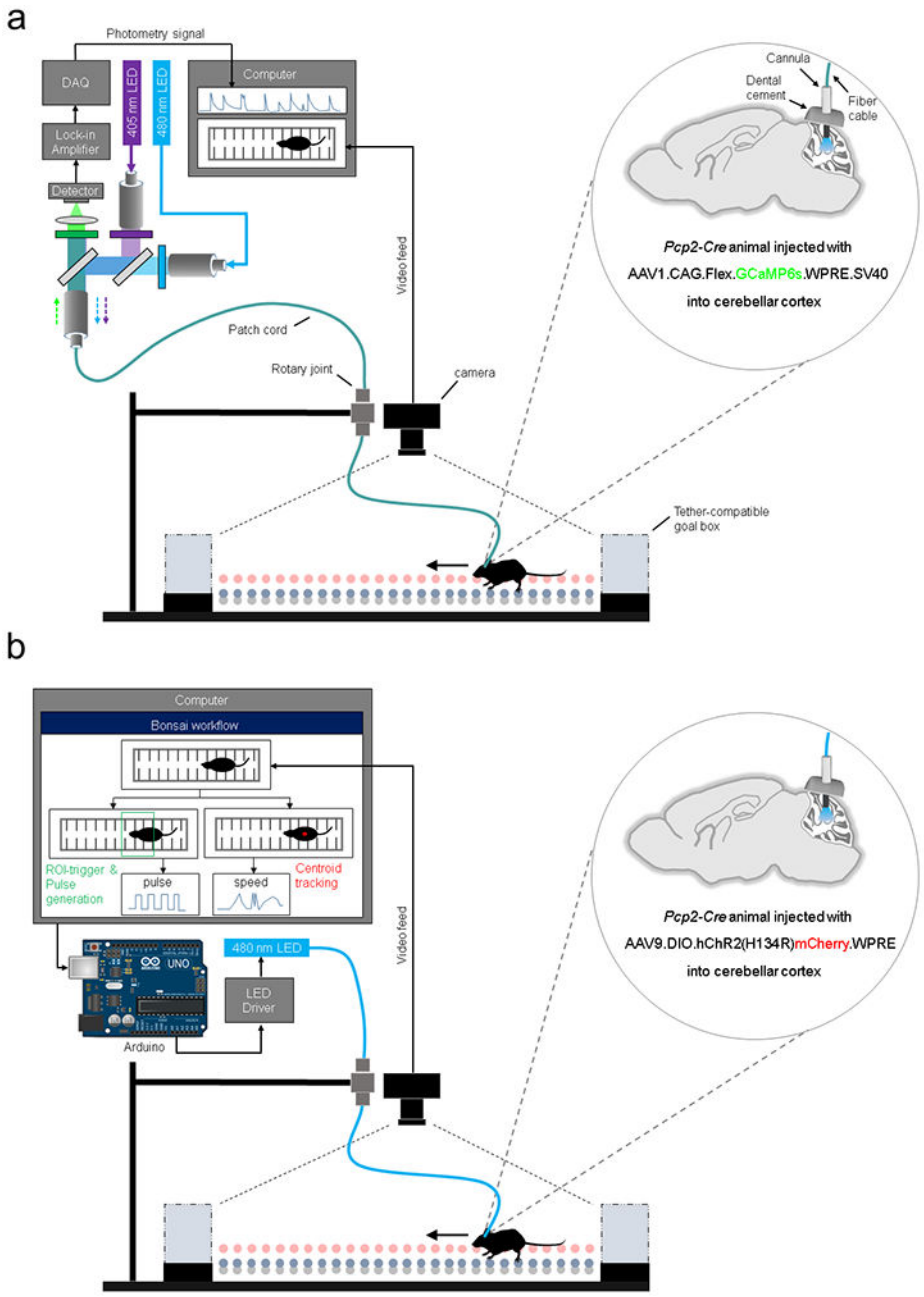

Figure 5. Tether-compatible experiments using the Erasmus Ladder to monitor or disrupt PC activity during locomotor behavior and cerebellar learning.

(a) Ca^{2+} fiber photometry set up in conjunction with an Erasmus Ladder experiment. A PC-specific Cre driver line (*Pcp2-Cre*) can be used to drive expression of GCaMP6s fluorescence indicator. Since the injected animals are implanted with a cannula, we can access the injection site by coupling it to a patch fiber optic cord, which then is a mode to excite GCaMP6s using a 480 nm (blue) LED and detect the fluorescence using a previously reported optical setup and design (Lerner, Shilyansky, Davidson, Evans, Beier, Zalocusky, Crow, Malenka, Luo, Tomer, and Deisseroth, 2015). The 405 nm LED (purple) is used as an internal control to subtract motion artifacts. A video camera capturing behavior can be used

to correlate position on the ladder to Ca^{2+} activity (b) Optogenetics in conjunction with an Erasmus Ladder experiment. *Pcp2-Cre* animals injected with a Channelrhodopsin AAV can drive robust expression in PCs. While the animal is behaving on the ladder, an independent platform running a Bonsai workflow with a live-video monitoring feed can be used to trigger optostimulation using an LED driver. Note that in both experimental designs, a tether-compatible goal box is required.