Cerebellar Outputs in Non-human Primates: An Anatomical Perspective Using Transsynaptic Tracers

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Andreea C. Bostan and Peter L. Strick

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Abstract

Important insights into cerebellar function can be gained from an anatomical analysis of cerebellar output. Recent studies using transsynaptic tracers in non-human primates demonstrate that the output of the cerebellum targets multiple nonmotor areas in the prefrontal and posterior parietal cortex, as well as the motor areas of the cerebral cortex. The projections to different neocortical areas originate from distinct output channels within the cerebellar nuclei. The neocortical area that is the main target of each output channel is a major source of input to the channel. Thus, a closed-loop circuit represents the fundamental macro-architectural unit of cerebro-cerebellar interactions. The outputs of these circuits provide the cerebellum with the anatomical substrate to influence the control of movement and cognition. Similarly, it has been shown that discrete multisynaptic loops connect the basal ganglia with motor and nonmotor areas of the cerebral cortex. Interactions between cerebro-cerebellar and cerebro-basal ganglia loops have been thought to occur mainly at the level of the neocortex. More recently,

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M. U. Manto et al. (eds.), *Handbook of the Cerebellum and Cerebellar Disorders*,
https://doi.org/10.1007/978-3-030-23810-0_25

neuroanatomical studies demonstrate that the anatomical substrate exists for substantial interactions between the cerebellum and the basal ganglia in both the motor and nonmotor domains. These data, along with the revelations about cerebro-cerebellar circuitry, provide a new framework for exploring the contribution of the cerebellum to diverse aspects of behavior.

Keywords

Cerebellar cortex · Rabies virus · Posterior parietal cortex · Cerebellar nucleus · Output channel

Introduction

The neocortical areas that provide inputs to the cerebellum have been well established (Fig. 1) (Glickstein et al. 1985; Schmahmann 1996). On the other hand, the targets of cerebellar output are still in the process of being fully identified (Strick et al. 2009). Recent results from neuroanatomical studies using transsynaptic tracers in nonhuman primates indicate that cerebellar output targets both motor and nonmotor areas of the cerebral cortex. This feature of cerebellar output provides part of the neural substrate for the involvement of cerebellum not only in the generation and control of movement but also in nonmotor aspects of behavior.

This chapter reviews new evidence about the areas of the cerebral cortex that are the target of cerebellar output. It describes the functional map that has recently been discovered within one of the major output nuclei of the cerebellum, the dentate nucleus. Furthermore, the chapter presents evidence that the fundamental unit of cerebro-cerebellar operations is a closed-loop circuit. Finally, it discusses the new anatomical evidence that the cerebellum and basal ganglia are interconnected.

The classical view of cerebro-cerebellar interconnections is that the cerebellum receives information from widespread neocortical areas, including portions of the frontal, parietal, temporal, and occipital lobes (Fig. 1) (Glickstein et al. 1985; Schmahmann 1996). This information was then thought to be funneled through cerebellar circuits where it ultimately converged on the ventrolateral nucleus of the thalamus (e.g., Allen and Tsukahara 1974; Brooks and Thach 1981). The ventrolateral nucleus was believed to project to a single neocortical area, the primary motor cortex (M1). Thus, cerebellar connections with the cerebral cortex were viewed as means of collecting information from widespread regions of the cerebral cortex. The cerebellum was thought to perform a sensorimotor transformation on its inputs and convey the results to M1 for the generation and control of movement. According to this view, cerebellar output was entirely within the domain of motor control, and abnormal activity in this circuit would lead to purely motor deficits.

Recent analysis of cerebellar output and function has challenged this view (e.g., Schell and Strick 1984; Middleton and Strick 1994, 1996a, b, 2000, 2001; Hoover and Strick 1999; Clower et al. 2001, 2005; Dum and Strick 2003; Kelly and Strick 2003; Akkal et al. 2007; Strick et al. 2009). It is now clear that efferents from the

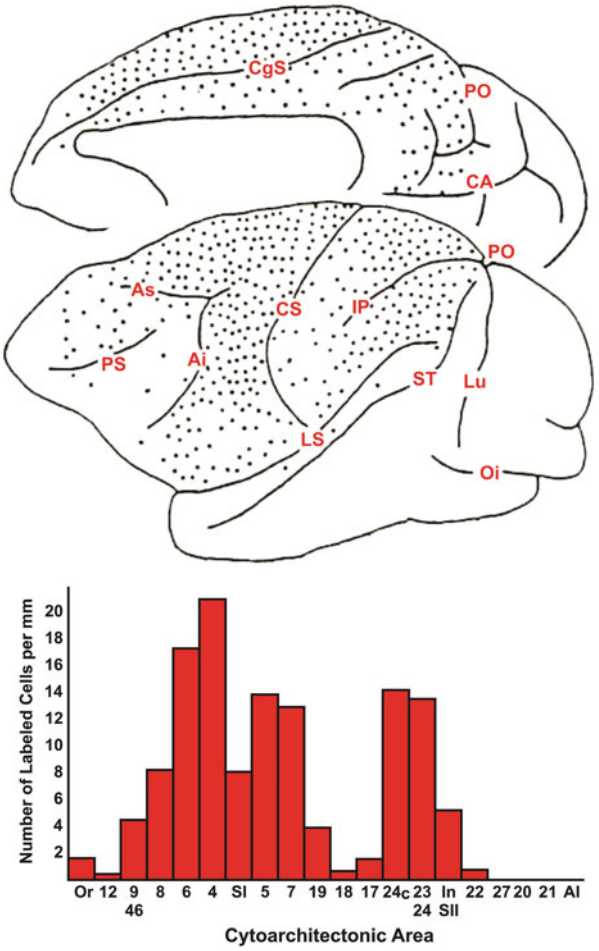

Fig. 1 Origin of projections from the cerebral cortex to the cerebellum. *Top*: The relative density of corticopontine neurons is indicated by the dots on the lateral and medial views of the macaque brain. *Bottom*: Histogram of relative density of corticopontine cells in different cytoarchitectonic areas of the monkey. *Ai*, *As* inferior and superior limbs of arcuate sulcus, respectively, *CA* calcarine fissure, *CgS* cingulate sulcus, *CS* central sulcus, *IP* intraparietal sulcus, *LS* lateral sulcus, *Lu* luneate sulcus, *IO* inferior occipital sulcus, *PO* parietal-occipital sulcus, *PS* principal sulcus, *STS* superior temporal sulcus (Adapted from Strick et al. (2009))

cerebellar nuclei project to multiple subdivisions of the ventrolateral thalamus (for a review, see Percheron et al. 1996), which, in turn, project to a myriad of neocortical areas, including regions of frontal, prefrontal, and posterior parietal cortex (Jones 1985). Thus, the outputs from the cerebellum influence more widespread regions of the cerebral cortex than previously recognized. This change in perspective is important because it provides the anatomical substrate for the output of the cerebellum to influence nonmotor as well as motor areas of the cerebral cortex. As a consequence, abnormal activity in cerebro-cerebellar circuits could lead not only to motor deficits but also to cognitive, attentional, and affective impairments.

Prior neuroanatomical approaches for examining cerebro-cerebellar circuits have been hindered by a number of technical limitations. Chief among these limitations is the multisynaptic nature of these pathways and the general inability of conventional

tracers to label more than the direct inputs and outputs of an area. To overcome these and other problems, neurotropic viruses have been used as transneuronal tracers in the central nervous system of primates (for references and a review, see Strick and Card 1992; Kelly and Strick 2000, 2003). Selected strains of virus move transneuronally in either the retrograde or anterograde direction (Zemanick et al. 1991; Kelly and Strick 2003). Thus, one can examine either the inputs to or the outputs from a site. The viruses used as tracers move from neuron to neuron exclusively at synapses, and the transneuronal transport occurs in a time-dependent fashion. Careful adjustment of the survival time after a virus injection allows for the study of neural circuits composed of two or even three synaptically connected neurons. Virus tracing has been used to examine cerebello-thalamocortical pathways to a wide variety of neocortical areas (Middleton and Strick 1994, 1996a, b, 2001; Lynch et al. 1994; Hoover and Strick 1999; Clower et al. 2001, 2005; Kelly and Strick 2003; Akkal et al. 2007) (Fig. 2).

Cerebellar Output Channels

In an initial series of studies, virus was injected into physiologically defined portions of M1 and the survival time was set to label second-order neurons in the deep cerebellar nuclei (Hoover and Strick 1999). In general, cerebellar projections to M1 originate largely from neurons in the dentate nucleus (75%), although a smaller component also originates from the interpositus (25%). Several studies have focused on the organization of the dentate nucleus. The dentate nucleus is a complex three-dimensional structure (Fig. 3). Results from different experiments can be displayed in a common framework on an unfolded map of the nucleus (Fig. 4) (Dum and Strick 2003).

Virus transport following injections into the arm representation of M1 labeled a compact cluster of neurons in the dorsal portion of the dentate at mid-rostro-caudal levels (Figs. 2 and 3, far right panel, Fig. 4, top center panel). Virus transport from the leg representation of M1 labeled neurons in the rostral pole of the dorsal dentate (Figs. 2 and 4, top left panel), whereas virus transport from the face representation labeled neurons at caudal levels of the dorsal dentate (Figs. 2 and 4, top right panel). Clearly, each neocortical area receives input from a spatially separate set of neurons in the dentate, which has been termed an output channel (Middleton and Strick 1997). The rostral to caudal sequence of output channels to the leg, arm, and face representations in M1 (Fig. 4, top panels, Fig. 5) corresponds well with the somatotopic organization of the dentate previously proposed on the basis of physiological studies (e.g., Allen et al. 1978; Stanton 1980; Rispal-Padel et al. 1982; Asanuma et al. 1983; Thach et al. 1993).

The region of the dentate that contains neurons that project to M1 occupies only 30% of the nucleus (Hoover and Strick 1999; Dum and Strick 2003). This implies that a substantial portion of the dentate projects to neocortical targets other than M1. To test this proposal and define the neocortical targets of the unlabeled regions of the

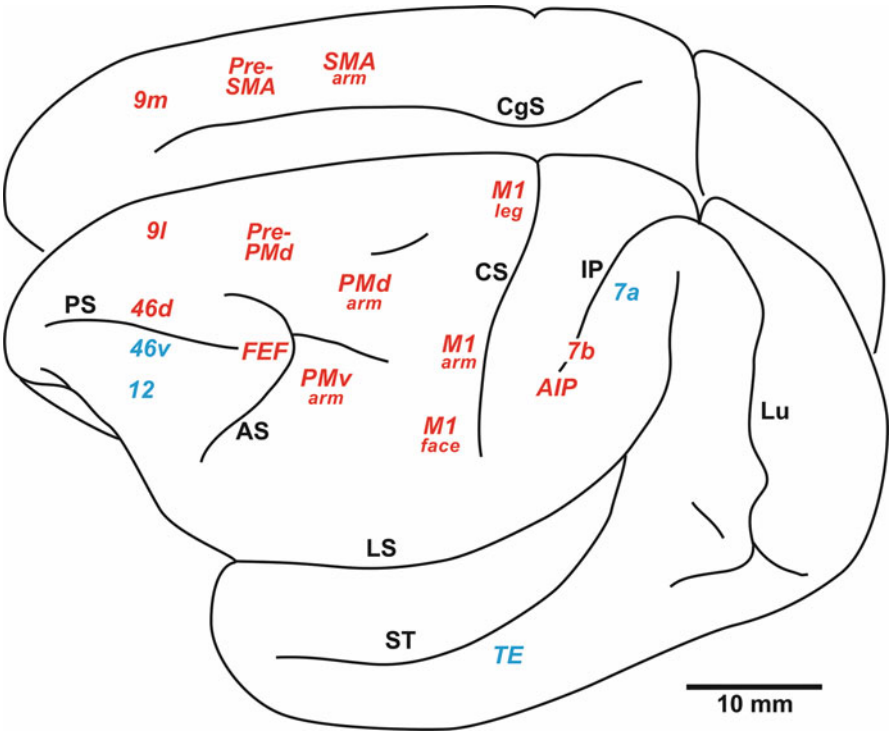

Fig. 2 Targets of cerebellar output. *Red labels* indicate areas of the cerebral cortex that are the target of cerebellar output. *Blue labels* indicate areas that are not the target of cerebellar output. These areas are indicated on lateral and medial views of the cebus monkey brain. The numbers refer to cytoarchitectonic areas. *AIP* anterior intraparietal area, *AS* arcuate sulcus, *CgS* cingulate sulcus, *FEF* frontal eye field, *IP* intraparietal sulcus, *LS* lateral sulcus, *Lu* lunate sulcus, *M1* face, arm, and leg areas of the primary motor cortex, *PMd arm* arm area of the dorsal premotor area, *PMv arm* arm area of the ventral premotor area, *PrePMd* predorsal premotor area, *PreSMA* presupplementary motor area, *PS* principal sulcus, *SMA arm* arm area of the supplementary motor area, *ST* superior temporal sulcus, *TE* area of inferotemporal cortex (Adapted from Strick et al. (2009))

dentate, virus was injected into selected premotor, prefrontal, and posterior parietal areas of the cortex (Fig. 2).

Virus transport from the arm representations of the ventral premotor area (PMv) and the supplementary motor area (SMA) provided evidence that both neocortical areas are the targets of cerebellar output (Fig. 2) (Middleton and Strick 1997; Akkal et al. 2007). The output channels to these premotor areas are located in the same region of the dentate that contains the output channel to arm M1 (Figs. 3 and 5). It has been hypothesized that the clustering of output channels to M1 and the premotor areas in the dorsal region of the dentate creates a motor domain within the nucleus (Fig. 5) (Dum and Strick 2003). It has been shown that the dorsal premotor cortex (PMd) also receives inputs from the motor territory of the dentate (Hashimoto et al. 2010). Interestingly, the output channels to the arm representations

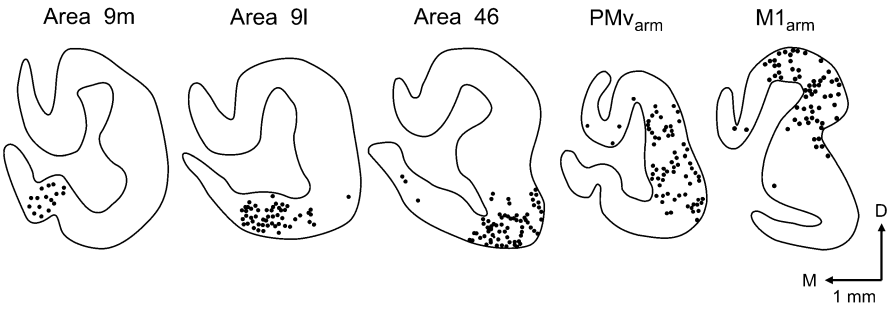

Fig. 3 Output channels in the dentate. The *dots* on representative coronal sections show the location of dentate neurons that project to a specific area of the cerebral cortex in the cebus monkey. The neocortical target is indicated above each section. Abbreviations are according to Fig. 2 (*M1* primary motor cortex, *PMv* ventral premotor area). *D* dorsal, *M* medial (Adapted from Middleton and Strick (1996b))

of M1, PMv, PMd, and SMA appear to be in register within the dentate. This raises the possibility that the nucleus contains a single integrated map of the body within the motor domain.

Virus transport following injections into prefrontal cortex revealed that some subfields are the target of dentate output, whereas others are not (Middleton and Strick 1994, 2001). Dentate output channels project to areas 9 m, 9 l, and 46d, but not to areas 12 and 46v (Figs. 2–5). Importantly, the extent of the dentate that is occupied by an output channel to a specific area of prefrontal cortex is comparable to that occupied by an output channel to a neocortical motor area (Fig. 4). Thus, it is likely that the signal from the dentate to prefrontal cortex is as important as its signal to one of the neocortical motor areas. In addition, dentate output channels to areas of prefrontal cortex are located in a different region of the nucleus than the output channels to the neocortical motor areas. The output channels to prefrontal cortex are clustered together in a ventral region of the nucleus that is entirely outside the motor domain. The output channels to prefrontal cortex are also rostral to the output channel that targets the frontal eye field (Lynch et al. 1994).

Although the presupplementary motor area (PreSMA) has traditionally been included with the motor areas of the frontal lobe, evidence indicates that it should be considered a region of prefrontal cortex (for reviews, see Picard and Strick 2001; Akkal et al. 2007). In support of this proposal, virus transport from the PreSMA labeled an output channel in the ventral dentate where the output channels to areas 9 and 46 are located (Figs. 2 and 4, bottom, Fig. 5). This result illustrates that the topographic arrangement of output channels in the dentate does not mirror the arrangement of their targets in the cerebral cortex. For example, the PreSMA is adjacent to the SMA on the medial surface of the hemisphere (Fig. 2), but the output channels to the two neocortical areas are spatially separated from one another in the dentate (Fig. 5). Thus, the topographic arrangement of output channels in the dentate appears to reflect functional relationships between neocortical areas rather than the spatial relationships among them.

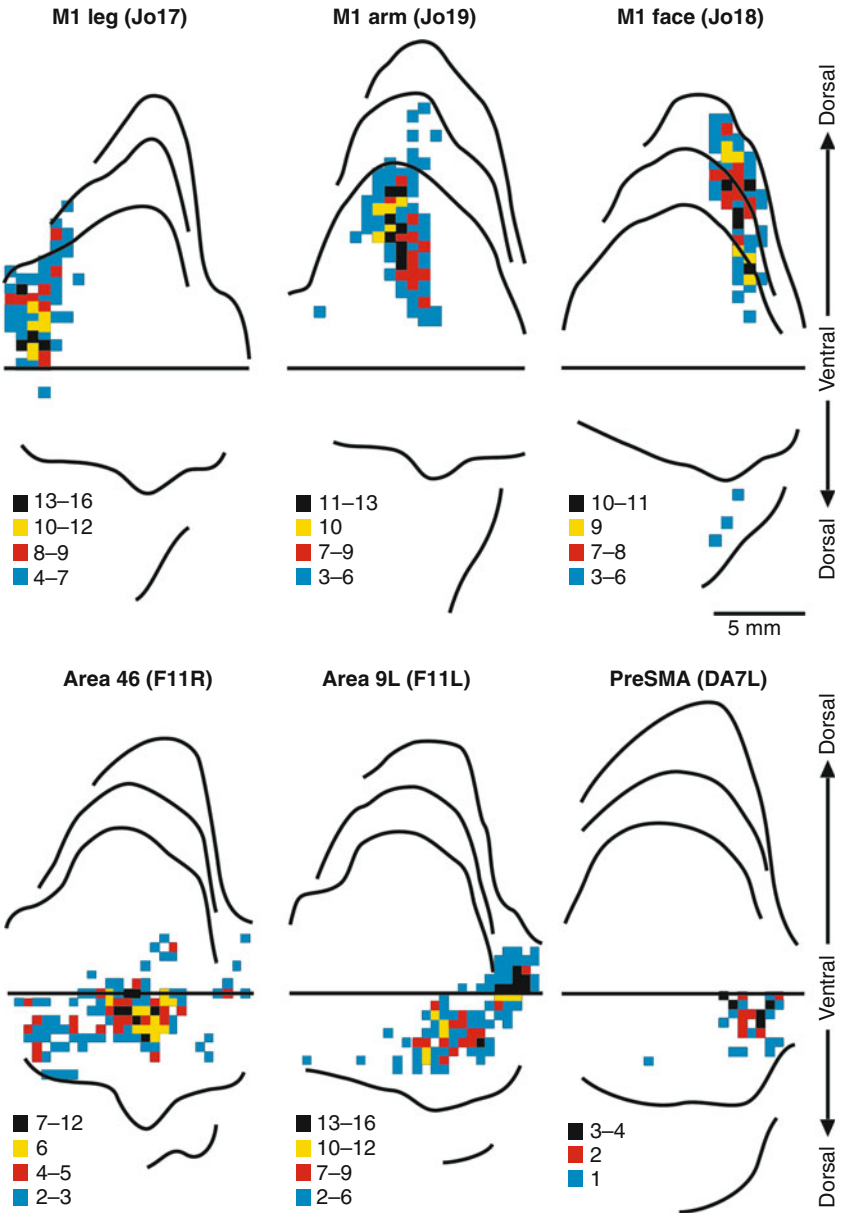

Fig. 4 Unfolded maps of the dentate: output channels to different areas of the cerebral cortex in the cebus monkey. *Top panels*: Somatotopic organization of output channels to leg, arm, and face M1 in the dorsal dentate. *Bottom panels*: Ventral location of output channels to prefrontal cortex. The key below each diagram indicates the density of neurons in bins through the nucleus. Rostral is to the left. Abbreviations are according to Fig. 1 (*M1* primary motor cortex, *PreSMA* presupplementary motor area) (Adapted from Dum and Strick (2003) (which includes a detailed description of the unfolding method))

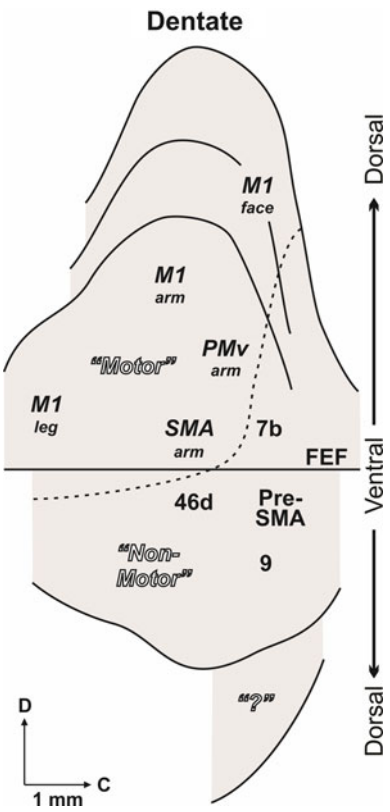

Fig. 5 Summary map of dentate topography. The lettering on the unfolded map indicates the neocortical target of different output channels in the cebus monkey. The location of different output channels divides the dentate into motor and nonmotor domains. Staining for monoclonal antibody 8B3 is most intense in the nonmotor domain. The *dashed line* marks the limits of intense staining for this antibody. The designation of the region marked by "?" is unclear. Abbreviations as in Fig. 2 (*FEF* frontal eye field, *M1* primary motor cortex, *PMv* ventral premotor area, *Pre-SMA* presupplementary motor area, *SMA* supplementary motor area) (Adapted from Dum and Strick (2003) and Akkal et al. (2007))

Virus transport from regions of posterior parietal cortex demonstrated that some of its subfields are also the target of output channels located in the dentate (Figs. 2 and 5) (Clower et al. 2001, 2005). For example, area 7b, which in the cebus monkey is located laterally in the intraparietal sulcus, is the target of an output channel located ventrally in the caudal pole of the dentate (Fig. 5). A second region of posterior parietal cortex, the anterior intraparietal area (AIP), receives a focal projection from a small cluster of neurons that is located dorsally in the dentate at mid-rostro-caudal levels. In addition, the AIP receives a broadly distributed projection from neurons that are scattered in dentate regions that contain output channels to M1, the PMv, and perhaps other premotor areas. This creates a unique situation in which AIP may receive a sample of the dentate output that is streaming to motor areas in the frontal lobe, as well as input from its own separate output channel. However, area 7a, which is located on the inferior parietal lobule (Fig. 2), does not receive substantial input from the dentate or other cerebellar nuclei (Clower et al. 2001). There also is evidence that the medial intraparietal area (MIP) and ventral lateral intraparietal area (LIPv) are the targets of cerebellar output from the deep cerebellar nuclei (Prevosto et al. 2010). Currently, the information about cerebellar

projections to other areas in the posterior parietal cortex is complex and incomplete. It is clear, however, that multiple areas of the posterior parietal cortex are the targets of output channels from the ventral dentate.

Maps from individual experiments have been coalesced into a single summary diagram where the average location of each output channel is indicated (Fig. 5). This summary diagram emphasizes several notable features about the topographic organization of the dentate. A sizeable portion of the nucleus projects to parts of the prefrontal and posterior parietal cortex. The output channels to prefrontal and posterior parietal areas are clustered in a ventral and caudal region of the nucleus. Consequently, these output channels are spatially segregated from those in the dorsal dentate that target motor areas of the cortex. Thus, the dentate appears to be spatially subdivided into separate motor and nonmotor domains that focus on functionally distinct neocortical systems. Another feature emphasized by the summary diagram is that the neocortical targets for large portions of the dentate remain to be determined.

The division of the dentate into separate motor and nonmotor domains is reinforced by underlying molecular gradients within the nucleus (Fortin et al. 1998; Pimenta et al. 2001; Dum et al. 2002; Akkal et al. 2007). Fortin et al. (1998) reported that immuno-staining for two calcium-binding proteins, calretinin and parvalbumin, is greatest in ventral regions of the squirrel monkey dentate. A monoclonal antibody, 8B3, which recognizes a chondroitin sulfate proteoglycan on subpopulations of neurons, also differentially stains the dentate in cebus monkeys and macaques (Pimenta et al. 2001; Dum et al. 2002; Akkal et al. 2007). Immunoreactivity for 8B3 is most intense in ventral regions of the dentate that project to prefrontal and posterior parietal areas of cortex. In contrast, antibody staining is least intense in the dorsal regions of the nucleus that project to the neocortical motor areas. These observations suggest that 8B3 recognizes a significant portion of the nonmotor domain within the dentate. Measurements indicate that approximately 40% of the nucleus is intensely stained by 8B3. This analysis does not include the caudal portion of the dentate (marked by a "?" in Fig. 5) because this region does not stain intensely for 8B3 and its neocortical target remains to be determined. However, based on its location, it is likely that this caudal region projects to a nonmotor area of the cerebral cortex. If this is the case, then the nonmotor domain of the dentate may represent as much as 50% of the nucleus in the cebus monkey.

In the human, it has long been recognized that the dentate is composed of a dorsal, microgyric portion and a ventral, macrogyric portion (for references and illustration, see Voogd 2003). Compared with the microgyric dentate, the macrogyric dentate is reported to (a) develop later, (b) have smaller cells, (c) display a selective vulnerability in cases of neocerebellar atrophy, and (d) have a higher iron content. This last observation suggests that molecular gradients may exist within the human dentate as they do in the monkey dentate; however, this possibility remains to be tested. Comparative studies suggest that the dentate has expanded in great apes and humans relative to the other cerebellar nuclei (Matano et al. 1985). Furthermore, most of this increase appears to be due to an expansion in the relative size of the ventral half of the dentate (Matano 2001). This observation implies that the nonmotor functions of the dentate grow in importance in great apes and humans.

Macro-Architecture of Cerebro-Cerebellar Loops

The neocortical areas that are the target of cerebellar output also project via the pons to the cerebellar cortex (Glickstein et al. 1985; Schmahmann 1996). This observation suggests that cerebro-cerebellar connections may form a closed-loop circuit. This concept has been tested for a representative motor area (the arm area of M1) and a nonmotor area (area 46 in the prefrontal cortex) (Kelly and Strick 2003). The anatomical evidence indicates that a specific region of the cerebellar cortex both receives input from and projects to the same area of the cerebral cortex.

Retrograde transneuronal transport of rabies virus was used to define the Purkinje cells in cerebellar cortex that project to M1 or to area 46. The arm area of M1

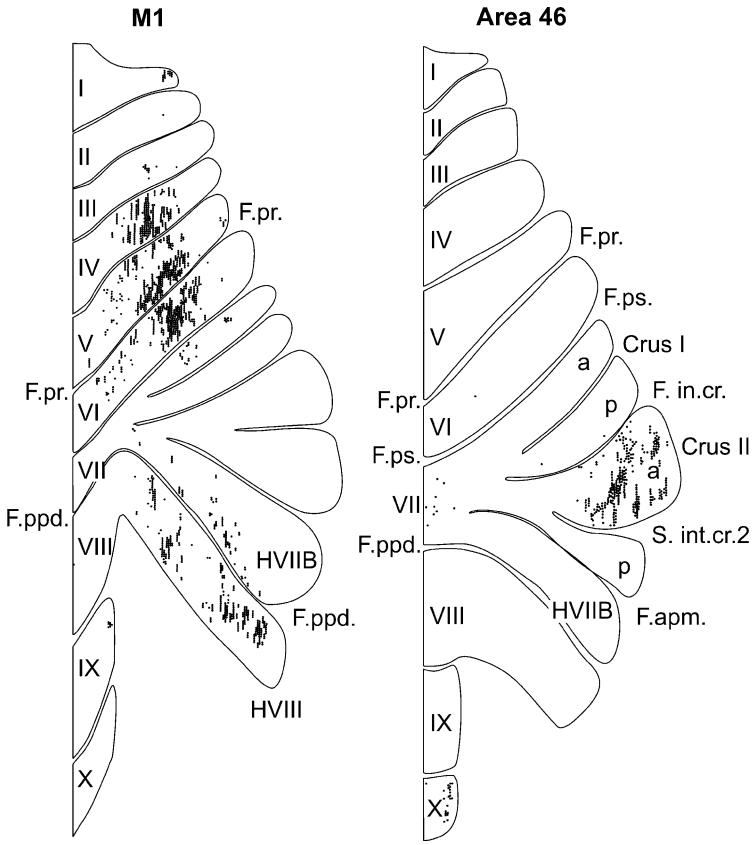

Fig. 6 Regions of cerebellar cortex that project to areas of cerebral cortex. The *black dots* on the flattened surface maps of the cerebellar cortex indicate the location of Purkinje cells that project to the arm area of M1 (*left panel*) or to area 46 (*right panel*) in the cebus monkey. The Purkinje cells that project to M1 are located in lobules that are separate from those that project to area 46. Nomenclature and abbreviations are according to Larsell (1970) (Adapted from Kelly and Strick (2003))

receives input from Purkinje cells located mainly in lobules IV–VI of the cerebellar cortex (Fig. 6, left panel). In contrast, area 46 receives input from Purkinje cells located mainly in Crus II of the ansiform lobule (Fig. 6, right panel). There is no evidence of overlap between the two systems. Thus, the two areas of the cerebral cortex are the targets of output from Purkinje cells that are located in separate regions of the cerebellar cortex. Clearly, the separation of motor and nonmotor functions seen in the dentate nucleus extends to the level of the cerebellar cortex.

In separate experiments, anterograde transneuronal transport of herpes virus was used to define the granule cells in cerebellar cortex that receive input from M1 or from area 46. The arm area of M1 projects to granule cells located mainly in lobules IV–VI, whereas area 46 projects to granule cells mainly in Crus II. Again, each cerebral cortical area projects to granule cells that are located in a separate region of the cerebellar cortex. Moreover, these findings indicate that the regions of the cerebellar cortex that receive input from M1 are the same as those that project to M1. Similarly, the regions of the cerebellar cortex that receive input from area 46 are the same as those that project to area 46. Thus, M1 and area 46 form separate, closed-loop circuits with different regions of the cerebellar cortex (Fig. 7). Altogether, these observations suggest that multiple closed loop circuits represent a fundamental macro-architectural feature of cerebro-cerebellar interactions.

There are a number of important functional implications to these results. They suggest that the cerebellar cortex is not functionally homogeneous. Instead, the results imply that cerebellar cortex contains localized regions that are interconnected with specific motor or nonmotor areas of the cerebral cortex. In fact, it has been hypothesized that the map of function in the cerebellar cortex is likely to be as rich and complex as that in the cerebral cortex (Kelly and Strick 2003). As a consequence, global dysfunction of the cerebellar cortex can cause wide-ranging effects on behavior (e.g., Schmahmann 2004). However, localized dysfunction of a portion of the cerebellar cortex can lead to more limited deficits, which may be motor or nonmotor depending on the specific site of the cerebellar abnormality (e.g., Fiez et al. 1992; Schmahmann and Sherman 1998; Allen and Courchesne 2003; Gottwald et al. 2004). Thus, precisely defining the location of a lesion, a site of activation, or a recording site is as important for studies of the cerebellum as it is for studies of the cerebral cortex.

As noted above, the neocortical targets for substantial portions of the dentate remain unidentified. In addition, fastigial and interpositus nuclei send efferents to the thalamus (Batton et al. 1977; Stanton 1980; Kalil 1981; Asanuma et al. 1983), and the neocortical targets of these deep nuclei remain to be fully determined. The closed-loop architecture described above enables us to make some predictions about additional neocortical targets of cerebellar output (Middleton and Strick 1998; Dum and Strick 2003; Kelly and Strick 2003). If closed-loop circuits reflect a general rule, then all of the areas of cerebral cortex that project to the cerebellum are the targets of cerebellar output. In addition to the neocortical areas that have already been investigated, the cerebellum receives input from a wide variety of higher-order, nonmotor areas. This includes areas of extrastriate cortex, posterior parietal cortex, cingulate cortex, and the parahippocampal gyrus on the medial

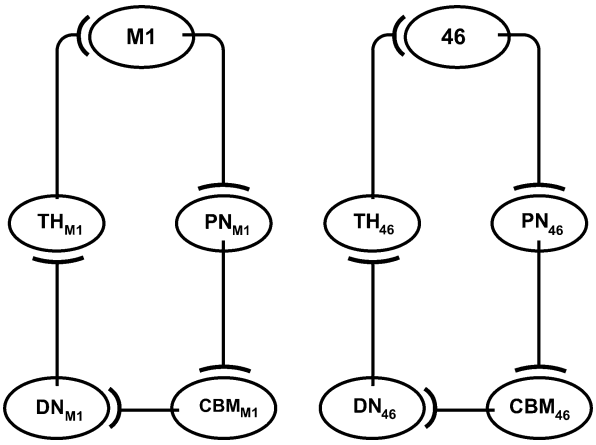

Fig. 7 Closed-loop circuits link the cerebellum with the cerebral cortex. Two topographically separate closed-loop circuits are illustrated. One interconnects the cerebellum with M1 and the other interconnects the cerebellum with area 46. In each loop, the neocortical area projects to a specific site in the pontine nuclei (PN), which then innervates a distinct region of the cerebellar cortex (CBM). Similarly, a portion of the dentate nucleus (DN) projects to a distinct region of the thalamus, which then innervates a specific neocortical area. Note that the neocortical area, which is the major source of input to a circuit, is the major target of output from the circuit. *CBM* cerebellar cortex, *DN* dentate, *PN* pontine nuclei, *TH* subdivisions of the thalamus (Adapted from Strick et al. (2009))

surface of the hemisphere (Fig. 1) (Brodal 1978; Wiesendanger et al. 1979; Vilensky and van Hoesen 1981; Leichnetz et al. 1984; Glickstein et al. 1985; Schmahmann and Pandya 1991, 1993, 1997). If some or all of these areas turn out to be cerebellar targets, then the full extent of cerebellar influence over nonmotor areas of the cerebral cortex is remarkable and much larger than previously suspected.

In discussing the neural substrate for a cerebellar influence over nonmotor functions, it is important to note the longstanding notion that the cerebellum is interconnected with the limbic system. Cerebellar stimulation can alter limbic function and elicit behaviors like sham rage, predatory attack, grooming, and eating (e.g., Zanchetti and Zoccolini 1954; Berntson et al. 1973; Reis et al. 1973). Cerebellar lesions can tame aggressive monkeys without creating gross motor abnormalities (Peters and Monjan 1971; Berman 1997). Classic electrophysiological evidence suggests that cerebellar stimulation, especially in portions of the fastigial nucleus and associated regions of vermal cortex, can evoke responses at limbic sites, including the cingulate cortex and amygdala (e.g., Anand et al. 1959; Snider and Maiti 1976). The major weakness in the cerebello-limbic hypothesis is the absence of a clear anatomical substrate that links the output of the cerebellum, and especially the fastigial nucleus, with limbic sites such as the amygdala. Although neuroanatomical evidence indicates that the deep cerebellar nuclei are interconnected with the hypothalamus (Haines et al. 1990), these connections do not appear sufficient to mediate all of the behavioral effects evoked by cerebellar stimulation. Thus, the circuits that link the output of the cerebellar nuclei with regions of the limbic system need to be explored using modern neuroanatomical methods.

The Cerebellum Is Interconnected with the Basal Ganglia

The loops that link the cerebellum with the cerebral cortex have traditionally been considered to be anatomically and functionally distinct from those that link the basal ganglia with the cerebral cortex (Doya 2000; Graybiel 2005). As the projections from the cerebellum and basal ganglia to the cerebral cortex are relayed through distinct thalamic nuclei (Percheron et al. 1996; Sakai et al. 1996), any interactions between cortico-cerebellar and cortico-basal ganglia loops were thought to occur primarily at the neocortical level. Results from recent anatomical experiments challenge this perspective and provide evidence for disynaptic pathways that link the cerebellum with the basal ganglia more directly.

To explore whether the cerebellum projects to the basal ganglia, rabies virus was injected into a region of the putamen. The injection sites were localized largely to the sensorimotor territory of the striatum (Parent and Hazrati 1995a). The virus went through two stages of transport: retrograde transport to first-order neurons in the thalamus that innervate the injection site and then, retrograde transneuronal transport to second-order neurons in the deep cerebellar nuclei that innervate the first-order neurons (Fig. 8). The neurons in the cerebellar nuclei that were labeled by virus transport were located largely in the dentate nucleus. Thus, a major output of cerebellar processing, the dentate, projects via the thalamus to an input stage of basal ganglia processing, the putamen.

In another series of experiments, rabies virus was injected into the external segment of the globus pallidus (GPe). The virus went through three stages of transport: retrograde transport of the virus from the injection site to first-order neurons in the striatum, retrograde transneuronal transport from these first-order neurons to second-order neurons in the thalamus, and retrograde transneuronal transport from the second-order neurons in the thalamus to third-order neurons in the deep cerebellar nuclei. Most of the labeled neurons in the cerebellar nuclei were confined to the dentate (Fig. 8). Thus, not only does the output from the cerebellum influence the striatum, but the target of this influence includes striatal neurons in the so-called indirect pathway which projects to GPe (e.g., DeLong and Wichmann 2007).

The injections of rabies virus into GPe involved two different regions of the nucleus. The injection in one animal labeled neurons primarily in ventral and caudal regions of dentate. The injection site in the other animal was placed approximately 1 mm caudally in GPe and labeled neurons in more dorsal regions of dentate. These observations suggest that the projection from the dentate to the basal ganglia is topographically organized. Virus transport from the basal ganglia labeled neurons in both the motor and nonmotor domains of the dentate (Hoshi et al. 2005). These observations suggest that the cerebellar projection to the input stage of basal ganglia processing influences motor and nonmotor aspects of basal ganglia function.

To explore whether the basal ganglia project to the cerebellum, rabies virus was injected into selected sites within the cerebellar cortex. The virus went through two stages of transport: retrograde transport of the virus from the injection site to first-order neurons in the pontine nuclei, and then, retrograde transneuronal transport

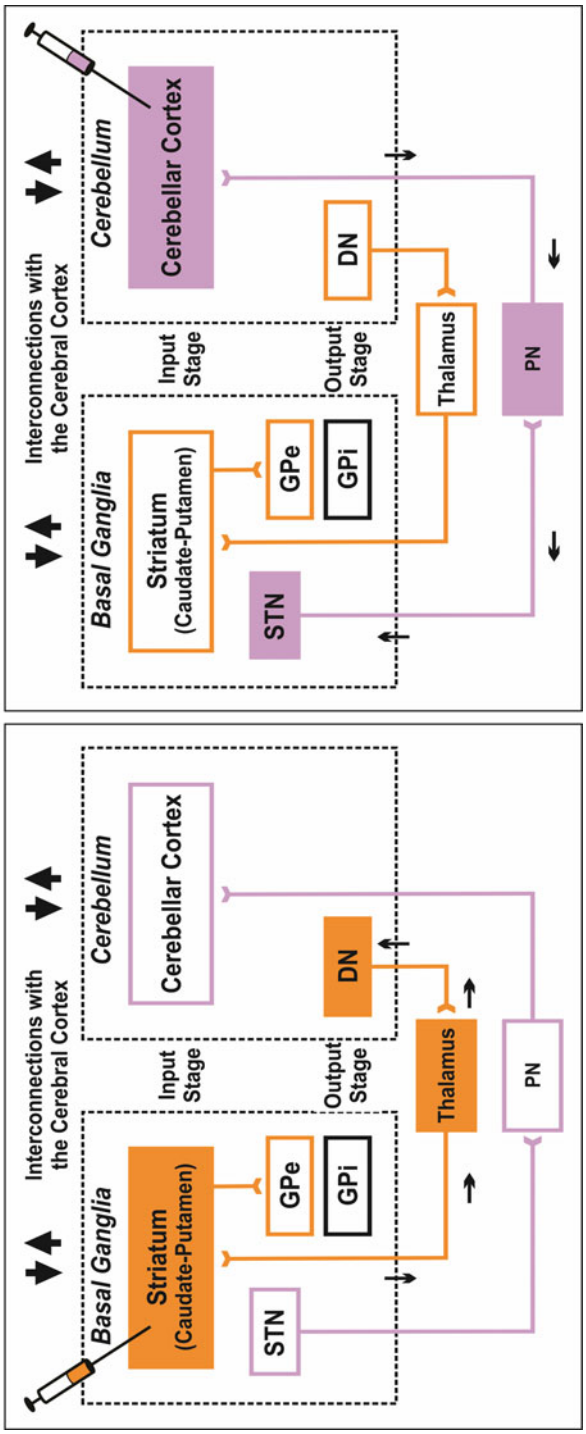

Fig. 8 Experimental paradigms and circuits interconnecting the cerebellum and basal ganglia: The *left panel* depicts the experimental paradigm and results from Hoshi et al. (2005), describing cerebellar output to the basal ganglia (*orange circuit*). Rabies virus was injected into the striatum. The virus went through two stages of transport: retrograde transport to first-order neurons in the thalamus that innervate the injection site and then, retrograde transneuronal transport to second-order neurons in the dentate nucleus (DN) that innervate the first-order neurons. Striatal neurons that receive cerebellar inputs include neurons in the "indirect" pathway that send projections to the external globus pallidum (GPe). The *right panel* of the figure depicts the experimental paradigm and results from Bostan et al. (2010), describing basal ganglia output to the cerebellum (*purple circuit*). Rabies virus was injected into the cerebellar cortex. The virus went through two stages of transport: retrograde transport to first-order neurons in the pontine nuclei (PN) that innervate the injection site and then, retrograde transneuronal transport to second-order neurons in the subthalamic nucleus (STN) that innervate the first-order neurons. These interconnections enable two-way communication between the basal ganglia and the cerebellum. Each of these subcortical structures has separate parallel interconnections with the cerebral cortex (*up and down large black arrows*). The *small black arrows* in both panels indicate the direction of virus transport. *DN* dentate nucleus, *GPe* external segment of the globus pallidus, *GPi* internal segment of the globus pallidus, *PN* pontine nuclei, *STN* subthalamic nucleus (Adapted from Bostan and Strick (2010))

from these first-order neurons to second-order neurons in the subthalamic nucleus (STN) of the basal ganglia (Figs. 8 and 9).

Rabies virus injections were placed in two areas within the hemispheric expansion of cerebellar lobule VII: the posterior aspects of Crus II (Crus IIp) and the hemispheric lobule VIIB (HVIIB). In all of these experiments, virus transport labeled substantial number of second-order neurons in the STN (Fig. 9). The second-order neurons labeled from virus injections into Crus IIp and HVIIB differed in their rostro-caudal and dorso-ventral distributions within the STN. The Crus IIp injections labeled larger numbers of neurons in ventromedial portions of rostral STN, whereas the HVIIB injections labeled larger numbers of neurons in the dorsal aspects of caudal STN (Fig. 9). Thus, a disynaptic connection links the STN with cerebellar cortex and this connection is topographically organized.

The STN can be subdivided into sensorimotor, associative, and limbic territories based on its interconnections with regions of the globus pallidus and the ventral pallidum (Fig. 9) (Parent and Hazrati 1995b; Joel and Weiner 1997; Hamani et al. 2004). The results from rabies virus injections into cerebellar cortex provide evidence that the projections from the STN to the cerebellar cortex originate from all three of its functional subdivisions. Specifically, most of the STN neurons that project to Crus IIp were found in the associative territory, in regions that receive substantial inputs from the frontal eye fields and regions of the prefrontal cortex (Fig. 9) (Monakow et al. 1978; Stanton et al. 1988; Inase et al. 1999; Kelly and Strick 2004). In contrast, most of the STN neurons that project to HVIIB were found in the sensorimotor territory, in regions that receive substantial inputs from the primary motor cortex and premotor areas of the frontal lobe (Fig. 9) (Monakow et al. 1978; Nambu et al. 1996, 1997; Inase et al. 1999; Kelly and Strick 2004). Therefore, the anatomical substrate exists for both motor and nonmotor aspects of basal ganglia processing to influence cerebellar function.

The results from the transsynaptic tracer studies reveal the anatomical substrate for two-way communication between the cerebellum and the basal ganglia in both the motor and nonmotor domains. One prediction from these findings is that activity in one of these major subcortical systems may directly affect the function of the other. Similarly, the interconnections between the two structures may enable abnormal activity at one site to propagate to the other.

Such interactions between the cerebellum and the basal ganglia are likely to have important implications for motor and nonmotor functions. They supply a framework for understanding cerebellar contributions to disorders such as Parkinson's disease and dystonia that have traditionally been considered "basal ganglia disorders" (for a review, see Bostan and Strick 2010). Furthermore, the anatomical connections between the cerebellum and the basal ganglia provide a potential explanation for the presence of cerebellar involvement in studies that were explicitly designed to study the normal functions of the basal ganglia. For example, several imaging studies have examined whether regions of the basal ganglia and related neocortical areas display functional activation consistent with their involvement in temporal difference models of reward-related learning (O'Doherty et al. 2003; Seymour et al. 2004). It is noteworthy that robust cerebellar activation was present in these

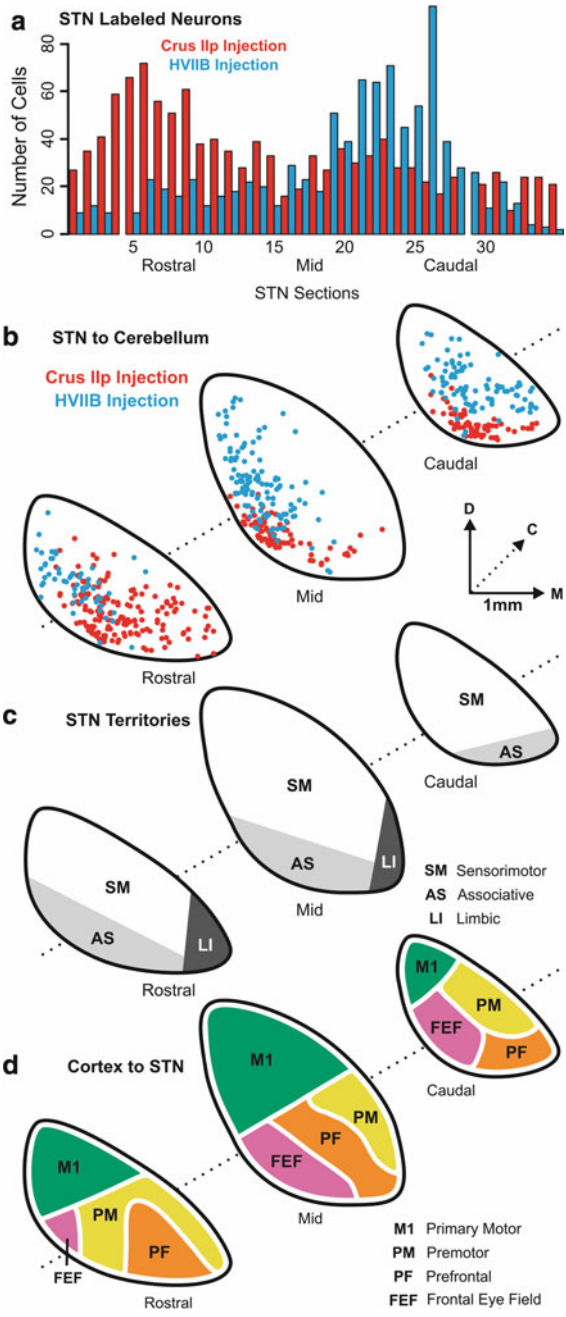

Fig. 9 STN projection to the cerebellar hemisphere: (**a**) Histogram of the rostro-caudal distribution of second-order neurons labeled in the STN by retrograde transport of virus from Crus IIp (*red bars*) and HVIIB (*blue bars*). *Missing bars* correspond to missing sections. (**b**) Charts of labeled neurons in STN after rabies virus injections into Crus IIp (*red dots*) and HVIIB (*blue dots*) are overlapped to illustrate the topographic differences in distribution of STN second-order neurons in the two cases. (**c**) Schematic representation of STN organization, according to the tripartite functional subdivisions of the basal ganglia (Parent and Hazrati 1995b; Joel and Weiner 1997; Hamani et al. 2004). (**d**) Schematic summary of the known connections between STN and areas of the cerebral cortex. *C* caudal, *D* dorsal, *M* medial, *STN* subthalamic nucleus (Adapted from Bostan et al. (2010))

experiments along with activation in the dorsal and ventral striatum. The disynaptic connection between the cerebellum and the basal ganglia provide an anatomical substrate for reward-related signals in the basal ganglia to influence cerebellar function during learning, and vice versa. Thus, the two subcortical structures may be linked together to form an integrated network. Future work is needed to elucidate the functional characteristics of this network.

Summary and Conclusions

The dominant view of cerebellar function over the past century has been that it is concerned with the coordination and control of motor activity through its connections with M1 (Brooks and Thach 1981). It is now apparent that a significant portion of the output of the cerebellum projects to nonmotor areas of the cerebral cortex, including regions of prefrontal and posterior parietal cortex. Thus, the anatomical substrate exists for cerebellar output to influence the cognitive and visuospatial computations performed in prefrontal and posterior parietal cortex (Clower et al. 2001, 2005; Middleton and Strick 2001). Furthermore, it has been shown that there are significant interconnections between the cerebellum and the basal ganglia in both the motor and nonmotor domains. Thus, the anatomical substrate exists for cerebellar output to influence the basal ganglia, and vice versa. As a corollary, abnormalities in cerebellar structure and function have the potential to produce multiple motor and nonmotor deficits by affecting various neocortical areas and subregions of the basal ganglia.

The output to nonmotor areas of the cerebral cortex and basal ganglia originates specifically from a ventral portion of the dentate. This nonmotor region of the dentate is recognized by several molecular markers. Several authors have argued that ventral dentate and related regions of the cerebellar hemispheres are selectively enlarged in great apes and humans (Leiner et al. 1991; Matano 2001). Indeed, the enlargement of the ventral dentate in humans is thought to parallel the enlargement of prefrontal cortex. These observations have led to the proposal that the dentate participation in nonmotor functions may be especially prominent in humans (e.g., Leiner et al. 1991; Schmahmann and Sherman 1998).

In recent years, concepts about cerebellar structure and function have changed radically. Not only is the cerebellum informed by neocortical information from multiple domains, but cerebellar output is directed at a variety of neocortical regions. As a consequence the output from the cerebellum can impact not only the generation and control of movement, but also cognition and affect. The anatomical evidence that the cerebellum exerts an influence over nonmotor function is complemented by results from neuroimaging studies and by the analysis of the deficits that accompany cerebellar lesions. Thus, it has become clear that the adaptive plasticity that the cerebellum provides for the generation and control of movement is also available for cognition and affect.

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