An Unfolded Map of the Cerebellar Dentate Nucleus and its Projections to the Cerebral Cortex

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ABSTRACT

We have used retrograde transneuronal transport of neurotropic viruses to examine the organization of the projections from the dentate nucleus of the cerebellum to motor and non-motor areas of the cerebral cortex. To perform this analysis we created an unfolded map of the dentate. Plotting the results from current and prior experiments on this unfolded map revealed important features about the topography of function in the dentate. We found that the projections to the primary motor and premotor areas of the cerebral cortex originated from dorsal portions of the dentate. In contrast, projections to prefrontal and posterior parietal areas of cortex originated from ventral portions of the dentate. Thus, the dentate contains anatomically separate and functionally distinct motor and non-motor domains.
INTRODUCTION

Traditionally, the dentate nucleus of the cerebellum was thought to project via the thalamus to a single cerebral cortical area, the primary motor cortex (M1) (e.g., Kemp and Powell 1971; Asanuma et al. 1983a; for references and review see Hoover and Strick 1999). According to this view, the output of the dentate was exclusively concerned with the control of movement. Recent studies have led to some significant alterations in this point of view. It is now clear that the dentate projection to M1 represents only a fraction of the output from the nucleus (Hoover and Strick 1999). Indeed, we have found that other portions of the dentate innervate oculomotor, prefrontal and posterior parietal areas of the cerebral cortex (Lynch et al. 1994; Middleton and Strick 1994, 2001; Clower et al. 2001). Furthermore, the projections to different cortical areas appear to originate from localized regions of the dentate which we termed output channels. This new anatomical data, along with results of behavioral and imaging studies (e.g., Ivry et al. 1988; Fiez et al. 1992; Mushiake and Strick 1993; Kim et al. 1994; Jueptner et al. 1997; Schmahmann 1997) have led to a reevaluation of dentate function (see the Special Issues of TINS 1998 and TICS 1998; Ramnani and Miall 2001).

The complex morphology of the dentate imposes a major barrier for unraveling the functional organization of the nucleus. To overcome this problem, we developed an unfolded map of the nucleus. This map enables the dentate's intricate structure to be viewed as a flat surface (Fig. 1). In general, unfolded maps have considerable heuristic value in their capacity to reveal organizational features obscured by complex three-dimensional structure (Brodal 1940; Larsell 1970; van Essen and Maunsell 1980; Dum and Strick 1991; He et al. 1995; van Essen and Drury 1997; van Essen et al. 2001). Unfolded maps also provide a link between the neuroanatomy and physiology of the non-human primate brain and that of the human brain (van Essen and Drury 1997; van Essen et al. 2001). In the present study, we
re-analyzed data from prior experiments at higher spatial resolution and replotted the results on our unfolded map of the dentate. These maps reveal a new perspective about the topographic organization of output channels within the dentate.

MATERIALS AND METHODS

Virus Tracing

The results of this study are based on an analysis of data from experiments in which the McIntyre-B strain of HSV1 was used as a retrograde transneuronal tracer to define the inputs to specific regions of motor, prefrontal and posterior parietal cortex (Hoover and Strick 1999; Middleton and Strick 2001; Clower et al. 2001). The procedures adopted for this study and the care provided experimental animals conformed to the regulations detailed in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols were reviewed and approved by the Institutional Animal Care and Use committees. The biosafety precautions taken during these experiments conformed to or exceeded the Biosafety Level 2 (BSL-2) regulations detailed in Biosafety in Microbiological and Biomedical Laboratories (Health and Human Services publication 93-8395). Detailed descriptions of the procedures for handling virus and virus-infected animals are presented in Strick and Card (1992) and Hoover and Strick (1999).

The details for these experiments have been described previously (Hoover and Strick 1999; Middleton and Strick 2001; Clower et al. 2001). Briefly, we injected virus into selected regions of the cerebral cortex of cebus monkeys (Cebus apella). The cortical regions injected were identified either by physiological mapping or by surface landmarks and their known relationship to the cytoarchitectonic borders of each cortical area. The locations of injections...
were later confirmed by cytoarchitectonic evaluation of the processed brain tissue. We used a post-injection survival time of 4-5 days. This survival time allows retrograde transport of HSV1 from the injection site to first-order neurons in the thalamus, and then retrograde transneuronal transport from these first-order neurons to second-order neurons in the dentate nucleus. At the end of the survival period, each animal was deeply anesthetized and perfused with aldehyde fixatives. The cerebellum was frozen and serially sectioned (50 um) in the coronal plane. To identify neurons infected with virus, we processed free-floating tissue sections according to the avidin biotin peroxidase method (Vectastain; Vector Laboratories, Burlingame, CA) using a commercially available antibody to HSV1 (Dako, Carpinteria, CA; 1:2000 dilution).

*Unfolded Map*

For the present study, we charted the outline of the dentate and the location of labeled neurons on every other serial section (for details, see Hoover and Strick 1999; Middleton and Strick 2001). Next, on each coronal section we drew a curved line through the nucleus midway between its medial and lateral outlines (Fig. 1A). The transitions between the major dentate segments and the position of each labeled neuron were projected onto the central line. Then, each line through the nucleus was unfolded and aligned on the transition between the c segment (the main vertical segment of the nucleus) and the d segment (the main ventral segment). This alignment minimizes the distortion of dentate regions that project to areas 4, 7b, 46 and 9l. To examine the density of labeled neurons we divided the unfolded lines into 200 um intervals and totaled the number of labeled neurons in each interval. The values of intervals from adjacent lines were combined to form 200 um X 200 um bins. A color code was assigned to each bin to indicate the number of labeled neurons it contained (Fig. 1C).
RESULTS

Neurons labeled by retrograde transneuronal transport of herpes simplex virus type 1 (HSV1) after injections into the arm representation of M1 were found dorsally in the dentate at mid rostrocaudal levels (Figs. 1B,C; 2B; 3A). Despite some slight differences in the injection sites (see Fig. 11 in Hoover and Strick 1999), the three animals with virus injections into the arm area displayed remarkably similar distributions of labeled neurons in the dentate (Figs. 1C, 2B, 3A). Because of this consistency, we used the origin of input to the arm area of M1 as the index case against which the origin of dentate projections to other cortical areas was compared.

In other animals, we placed injections of virus into the leg and face areas of M1 (defined by intracortical stimulation, see Figs. 12 and 13 in Hoover and Strick, 1999). Injections into the leg area also labeled neurons in a dorsal portion of the dentate, but in this case at more rostral levels of the nucleus (Fig. 2A). Likewise, injections into the face area labeled neurons dorsally in the dentate, but at more caudal levels of the nucleus (Fig. 2C). This rostral to caudal arrangement of the origin of projections to the leg, arm and face representations in M1 corresponds well with the somatotopy previously proposed for the dentate (e.g. Allen et al. 1978; Stanton 1980; Rispal-Padel et al. 1982; Asanuma et al. 1983b). In contrast, it should be clear from the unfolded maps that large portions of the dentate nucleus were not labeled following virus injections into M1. As a consequence, the body map generated by the projections to M1 occupied only a portion of the dorsal third of the nucleus.

Some of the dentate regions that were not labeled after virus injections into M1 did contain labeled neurons after injections into prefrontal and posterior parietal areas of the cortex (Middleton and Strick 2001; Clower et al. 2001). For example, virus injections into a part of
area 7b located in the intraparietal sulcus resulted in labeled neurons in ventral portion of caudal dentate (Fig. 3B). In contrast, virus injections into a part of area 46 located dorsal to the principal sulcus labeled neurons ventrally at mid rostro-caudal levels of the dentate (Fig. 3B). Virus injections into a part of area 9 located on the lateral surface of the hemisphere labeled neurons in yet another part of caudal dentate (Fig. 3D). Clearly, a substantial portion of the output from the dentate targets non-motor areas of cortex in a topographically organized manner (e.g., Middleton and Strick 1994, 2001; Clower et al. 2001).

**DISCUSSION**

Our results provide new insights into the topographic organization of dentate projections to the cerebral cortex. A summary of our findings is shown on a single unfolded map of the nucleus (Fig. 4A). Labels are placed at the sites in the dentate that project most densely to different cortical areas. The origin of projections to the arm representation of the ventral premotor area (PMv) is included in this diagram based on data from Middleton and Strick (1997) (see also Orioli and Strick 1989). This map emphasizes that the dentate is anatomically divided into separate output channels that project to distinct cortical areas.

The unfolded map also illustrates another important perspective about the topographic organization of dentate output-- a substantial portion of the nucleus projects to areas of prefrontal and posterior parietal cortex, in addition to its classical motor targets. Furthermore, the output channels that target motor areas of the cortex are segregated from those that target prefrontal and posterior parietal areas of the cortex. Thus, the nucleus appears to be functionally divided into separate motor and non-motor domains.

The motor domain in the dorsal portion of the dentate contains output channels
concerned with aspects of movement generation and control. Output neurons that influence leg, arm and face regions of M1 are arranged in a rostral to caudal sequence within the dorsal part of the nucleus (Fig. 4A, also see Hoover and Strick 1999). In addition, the motor domain contains distinct output channels that innervate a number of the premotor areas in the frontal lobe (Fig. 4A; Middleton and Strick 1997; Dum and Strick 1999; Akkal et al. 2001). Overall, our current estimate is that the motor domain in the monkey comprises approximately 50-60% of the dentate.

In contrast, the non-motor domain in the ventral portion of the dentate contains output channels concerned with aspects of cognition and visuo-spatial function. Within this region, neurons that project to prefrontal and posterior parietal cortical areas are clustered into distinct regions that display little evidence of overlap. Single neuron recordings in this area have seldom found neurons with changes in activity specifically related to limb movements (e.g., Thach 1978; Schieber and Thach 1985; Wett's et al. 1985). At least 20% of the volume of the dentate is occupied by output channels that innervate portions of areas 9, 46 and 7 (Middleton and Strick 2001; Clower et al. 2001). The cortical targets of the rest of the dentate remain to be determined. However, the amount of the dentate located in the non-motor domain is likely to grow as projections to other cortical regions are explored.

So far, all the cerebral cortical areas that are the target of dentate output also project back upon the cerebellum via efferents to pontine nuclei (i.e., Fig. 4B, shaded region) (Brodal 1978; Glickstein et al. 1985; Schmahmann 1998). Conversely, cortical areas that do not project back upon the cerebellum do not appear to be the target of dentate output (e.g., the ventral portion of area 46, the lateral portion of area 12 and area TE in inferotemporal cortex)(for discussion see Middleton and Strick 1996, 2001). These observations lead to the conclusion that multiple closed-loop circuits appear to be a major functional unit of cerebro-cerebellar
circuitry (Middleton and Strick 1998). This arrangement suggests that some of the unknown targets of dentate output are other non-motor areas that are known to provide input to the cerebellum (e.g., portions of area 5, area 7 and anterior and posterior cingulate cortex) (Brodal 1978; Glickstein et al. 1985; Schmahmann, 1998). We are currently testing this prediction.

Molecular markers may also prove useful for identifying the full extent of the non-motor domain of the dentate. Immuno-staining with 8B3, an antibody which recognizes a novel proteoglycan epitope localized on cell membranes, stains ventral regions of the macaque dentate more intensely than more dorsal regions (Pimenta et al. 2001). Similarly, immuno-staining for two calcium binding proteins, calretinin and parvalbumin, is reported to be greatest in ventral regions of the squirrel monkey dentate (Fortin et al. 1998). The precise correlation between these differential patterns of immuno-staining and the functional topography of the dentate remains to be determined. However, if a match exists, these molecular markers may provide a bridge for constructing homologies between the motor and non-motor domains in the monkey and human dentate.

*Mapping of Dentate Output Channels onto the Thalamus*

To understand how the output of the dentate maps upon the ventrolateral thalamus, we have displayed the origin of efferents to different cortical areas on a single section through the thalamus (Fig. 4C). Labels are placed at sites in the ventrolateral thalamus that receive cerebellar efferents and project to the cortical areas indicated. Although this map is a considerable simplification of the complex three-dimensional architecture of thalamocortical relationships, it has some important heuristic value. For example, the rostral to caudal topography in the dentate appears to translate into a lateral to medial topography in the thalamus. Within the motor domain of the dentate, the rostral -leg to caudal-face somatotopy
(Fig. 4A) is transformed into a lateral-leg to medial-face somatotopy in the ventrolateral thalamus (Fig. 4C; Strick 1976a,b; Kievit and Kuypers 1977; Miyata and Sasaki 1983; Vitek et al. 1994). A similar topographic mapping appears to be present between the output channels in the ventral, non-motor domain of the dentate and VLcc in the thalamus.

A comparison of the dentate and thalamic maps also illustrates that the dorsal to ventral topography in the dentate is inverted to form a ventral to dorsal topography in the thalamus. As a consequence, we have portrayed the thalamus upside down to maintain the correspondences between the maps (compare Fig. 4A to 4C). The motor and non-motor domains of the dentate project to distinct territories in the ventrolateral thalamus (Fig. 4A, C). Efferents from the dorsal, motor domain of the dentate project upon the classic motor subdivisions of the ventrolateral thalamus (e.g., VPLo and area X according to Olszewski 1952). In contrast, efferents from the ventral, non-motor domain terminate more dorsally in portions of VLcc (Fig. 4C, VLcc according to Holsapple et al. 1991). Thus, in both the dentate and VLcc, projections to widely dispersed areas of prefrontal and posterior parietal cortex have been collected together.

The dominant view of the cerebellum over the last century has been that it is concerned with the coordination and control of motor activity (Brooks and Thach 1981). It is now clear that the anatomical substrate exists for cerebellar output to influence non-motor as well as motor areas of the cerebral cortex (Middleton and Strick 1998; Hoover and Strick 1999; Middleton and Strick 2001; Clower et al. 2001). Our map shows that a significant portion of the output from the cerebellum is directed to cortical regions thought to be involved in cognitive and visuo-spatial functions (Middleton and Strick 2001; Clower et al. 2001). Indeed, ventral portions of dentate are activated during a variety of tasks involving short term working memory, rule based learning and higher executive function like planning (Mushiake and Strick 1993; Kim et al. 1994;
Jueptner et al. 1997; Liu et al. 2000). There is evidence that the proportion of dentate output to cortical regions involved in cognitive functions is larger in humans than monkeys (Leiner et al. 1991; Matano 2001). Thus, the dentate’s participation in non-motor functions may be expanded in the human (e.g., Schmahmann and Sherman 1998).

Our results along with recent physiological observations (Mushiake and Strick 1993; Kim et al. 1994; Jueptner et al. 1997; Liu et al. 2000) suggest that there is a topography of function within the dentate. The nucleus clearly contains separate motor and non-motor domains. Our unfolded map of the nucleus, like other flattened maps, provides a consistent anatomical framework for future explorations into the topography of function in the dentate.
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Figure Legends

FIG. 1. Unfolded map of the dentate nucleus. A: This map of the dentate was created by unfolding serial coronal sections through the nucleus. Typical sections are displayed around the map to indicate the shape of the dentate at selected levels. The unfolding process began by drawing a line through the center of the nucleus on each section. The color coded segments of these lines correspond to the colored regions on the unfolded map. Each section is aligned to the junction of the \( c \) and \( d \) segments (open star). The dashed vertical line indicates the rostro-caudal center of the nucleus. See Methods for further explanation. B: Cross-sections of the dentate nucleus showing the location of second order neurons (dots) labeled after transport of HSV1 from the arm representation of M1. C: The distribution and density of labeled neurons in the same animal are displayed on an unfolded map of the dentate. This map was reconstructed from every other coronal section.

FIG. 2. Somatotopic organization of dentate output channels to M1. Unfolded maps of the dentate illustrate the neurons labeled after HSV1 injections into the A: leg, B: arm, and C: face representations of M1. Conventions and abbreviations as in Fig. 1. The arm case shown in B is from a different animal than those illustrated in Figs. 1 and 3A.

FIG. 3. Dentate output channels to the prefrontal and posterior parietal cortex. Unfolded maps of the dentate illustrate the neurons labeled after HSV1 injections into A: the arm representation of M1, B: area 7b, C: area 46, and D: area 9l. Conventions and abbreviations as in Fig. 1. The arm case shown in A is from a different animal than those illustrated in Figs. 1 and 2B.
FIG. 4. Topography within the cerebello-thalamocortical circuit.  

A: Dentate output channels. The origins of the peak density of dentate projections to selected cortical areas are labeled.  

B: Selected cortical targets of cerebello-thalamocortical circuits. The shading in this diagram indicates the cortical regions (lateral hemisphere only) that project to the cerebellum via the pons (after Brodal 1978; Glickstein et al. 1985; Schmahmann 1998).  

C: Origin of selected cortical projections from the ventrolateral thalamus. The cortical regions indicated receive input from regions of the ventrolateral thalamus that lie within the termination zone of cerebellar efferents. The thalamus has been turned upside down to indicate the match between its topography and that of the dentate.  

ArS, arcuate sulcus; CM/Re, nucleus centrum medianum/nucleus reuniens; CS, central sulcus; IPS, intraparietal sulcus; MD, nucleus medialis dorsalis; PMv- ventral premotor area; PS, principal sulcus; VLcc, caudal portion of the nucleus ventralis lateralis, pars caudalis; VPI, nucleus ventralis inferior; VPLo, nucleus ventralis posterior lateralis, pars oralis; X, Area X.
Figure 1
Figure 2
Figure 4