BRIEF COMMUNICATIONS

nature neuroscience

The cerebellum communicates with the basal ganglia

Eiji Hoshi[1–3], Léon Tremblay[4], Jean Féger[4], Peter L Carras[1,2] & Peter L Strick[1,2,5]

The cerebral cortex is interconnected with two major subcortical structures: the basal ganglia and the cerebellum. How and where cerebellar circuits interact with basal ganglia circuits has been a longstanding question. Using transneuronal transport of rabies virus in macaques, we found that a disynaptic pathway links an output stage of cerebellar processing, the dentate nucleus, with an input stage of basal ganglia processing, the striatum.

The basal ganglia and cerebellum are two major subcortical structures that influence multiple aspects of motor, cognitive and affective behavior[1–5]. Both structures are densely interconnected with the cerebral cortex. For example, large numbers of cortical neurons project to the input stages of the basal ganglia (the caudate and putamen) and the cerebellum (the pontine nuclei). Similarly, the output stages of the basal ganglia (the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata) and the cerebellum (the deep cerebellar nuclei) project to subdivisions of the ventroanterior and ventrolateral thalamus[6,7]. These regions of the thalamus then project back upon the cerebral cortex. Thus, a major architectural feature of these circuits is the formation of multiple 'loops' between cerebral cortex and basal ganglia and between cerebral cortex and cerebellum. Basal ganglia and cerebellar loops are believed to operate largely in isolation from one another because the outputs from the two circuits project to neighboring, but separate, thalamic nuclei[6,7]. The major site for interaction between these circuits was thought to be at the level of the cerebral cortex. We now provide evidence for a pathway that enables the output stage of cerebellar processing to have a direct influence over the input stage of basal ganglia processing.

We injected the N2C strain of rabies virus into sites within the basal ganglia of six macaque monkeys (**Fig. 1**, **Supplementary Methods** and **Supplementary Fig. 1**). Rabies virus is transported transneuronally in a time-dependent fashion in the CNS of nonhuman primates[8]. We used this feature of the virus to determine if neurons in the deep cerebellar nuclei project to the basal ganglia and to define the links in this connection. All experimental procedures were approved by the institutional animal care and biosafety committees of the University of Pittsburgh and were in accordance with the regulations detailed in the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

In the first experiments, we injected a small amount of the N2C strain of rabies into the putamen (**Fig. 1a**, $n = 2$; **Supplementary Figs. 1**,**2**) and allowed the animals to survive for 40 h. With the N2C strain, 40 h is long enough for retrograde transport of the virus from the injection site to 'first-order' neurons and, subsequently, retrograde transneuronal transport to 'second-order' neurons that innervate the first-order neurons. After the putamen injections we observed retrograde transport of the virus to first-order neurons in the thalamus and then retrograde transneuronal transport from these first-order neurons to second-order neurons in the deep cerebellar nuclei. Although our putamen injections were relatively small and localized (**Fig. 1a**, **Supplementary Fig. 2**), we labeled an average of 149 neurons in the cerebellar nuclei. This number reflects counts from every other section through the cerebellum. Of these labeled neurons, 88% were located in the contralateral nuclei; 67% of the contralateral neurons were located in the dentate, 29% were in interpositus and 4% were in fastigial. The labeled neurons were most concentrated in dorsal and ventral portions of the rostral dentate. The morphology of these labeled neurons was typical of dentate neurons that project to cortex via the thalamus[9].

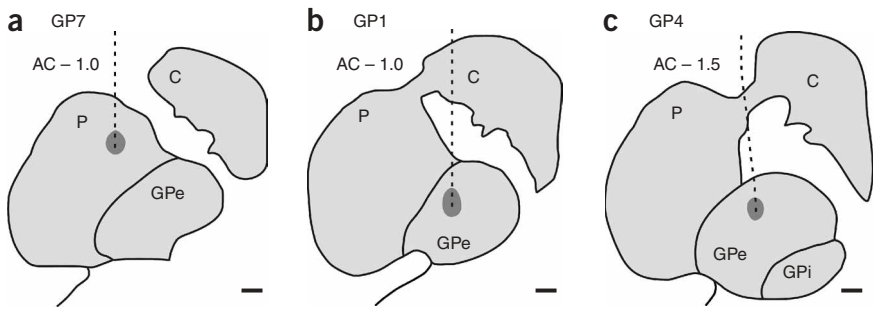

Figure 1 Tracer injection sites. Rabies virus (N2C strain) was injected into different locations within the basal ganglia: (**a**) putamen, animal GP7; (**b**,**c**) external segment of the globus pallidus (GPe), animals GP1 and GP4. The survival time was 40 h in **a** and **c** and 50 hours in **b** (**Supplementary Fig. 1**). The shaded ellipse in each panel indicates the injection site; the dashed line indicates the track of the injection cannula. Scale bars, 1 mm. AC, anterior commisure; C, caudate; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; P, putamen. AC – 1.0: 1.0 mm caudal to AC. AC – 1.5 mm: 1.5 mm caudal to AC.

[1]Department of Neurobiology and [2]Center for the Neural Basis of Cognition, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA. [3]Tamagawa University Research Institute, Machida, Tokyo 194-8610 Japan. [4]Neurologie et Thérapeutique expérimentale (INSERM U679), Hôpital de la Salpêtrière, 47 boulevard de l'Hôpital, 75651 Paris CEDEX 13, France. [5]Pittsburgh Veterans Affairs Medical Center, Pittsburgh, Pennsylvania 15261, USA. Correspondence should be addressed to P.L.S. (strickp@pitt.edu).

Received 26 April; accepted 22 August; published online 2 October 2005; doi:10.1038/nn1544

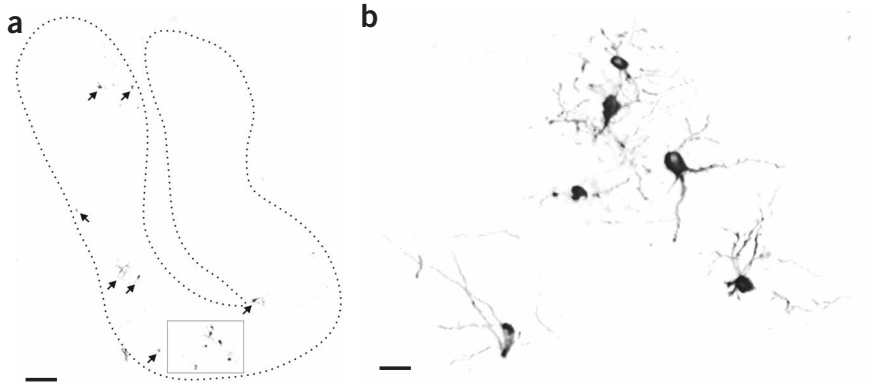

Figure 2 Dentate neurons labeled by retrograde transneuronal transport of virus from GPe. (**a**) Labeled neurons on a coronal section through the dentate nucleus. The animal was allowed to survive for 50 h after an injection of rabies into GPe. Each arrow points to a labeled neuron. Scale bar, 200 μm. (**b**) An enlargement of the boxed area in **a**. Scale bar, 50 μm.

In the second experiments, we injected a small amount of the N2C strain into the external segment of the globus pallidus (GPe) and allowed the animals to survive for 50 h (**Fig. 1b**, $n = 2$; **Supplementary Fig. 1**). Fifty hours is long enough for transneuronal transport of the N2C strain to 'third-order' neurons. After the GPe injections we observed 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 then another stage of retrograde transneuronal transport from these second-order neurons to third-order neurons in the deep cerebellar nuclei (**Figs. 2,3**). The small injections of virus into GPe labeled an average of nearly 1,400 neurons in the cerebellar nuclei, or approximately ten times the number of neurons labeled after similar-sized injections into the putamen. As with the putamen injections, 88% of the labeled neurons were located in the contralateral cerebellar nuclei; of these, 69% were located in the dentate, 14% were in interpositus and 17% were in fastigial. After the GPe injection (**Fig. 1b**), labeled neurons were most numerous in ventral and caudal regions of the dentate (**Fig. 3b**). In a second animal, an injection of virus was placed ~1 mm caudally in GPe, and the region with dense labeling shifted to more dorsal regions of the dentate.

We have previously presented evidence that the dentate contains distinct 'motor' and 'nonmotor' domains[10] (**Fig. 3c**). These regions of the nucleus contain neurons that project via the thalamus to primary motor, premotor, prefrontal and posterior parietal areas of the cerebral cortex[9–11]. The neurons labeled after virus injections into GPe (and into the putamen) were located in one or both of these domains (**Fig. 3b**). Furthermore, the number and density of dentate neurons labeled after GPe injections were comparable to the number and density of dentate neurons labeled after virus injections into some posterior parietal and prefrontal areas of cortex[9–11]. This result implies that the dentate influence on a stage of basal ganglia processing is as substantial as its influence on some areas of the cerebral cortex.

In the third experiments (**Fig. 1c**, $n = 2$; **Supplementary Fig. 1**), we injected a small amount of the N2C strain into GPe and allowed the animals to survive for 40 h. As noted above, this survival time is long enough for only one stage of retrograde transneuronal transport. After these injections into GPe we observed retrograde transport from the injection site to first-order neurons in the striatum and then retrograde transneuronal transport from these first-order neurons to second-order neurons in the thalamus. The labeled neurons in the thalamus were found in subdivisions of ventroanterior/ventrolateral thalamus and were particularly numerous in regions of several intralaminar nuclei including the paracentral, central lateral and centromedian-parafascicular complex[6] (**Supplementary Fig. 2**). In contrast, we found only one or two labeled neurons in the contralateral dentate of each animal.

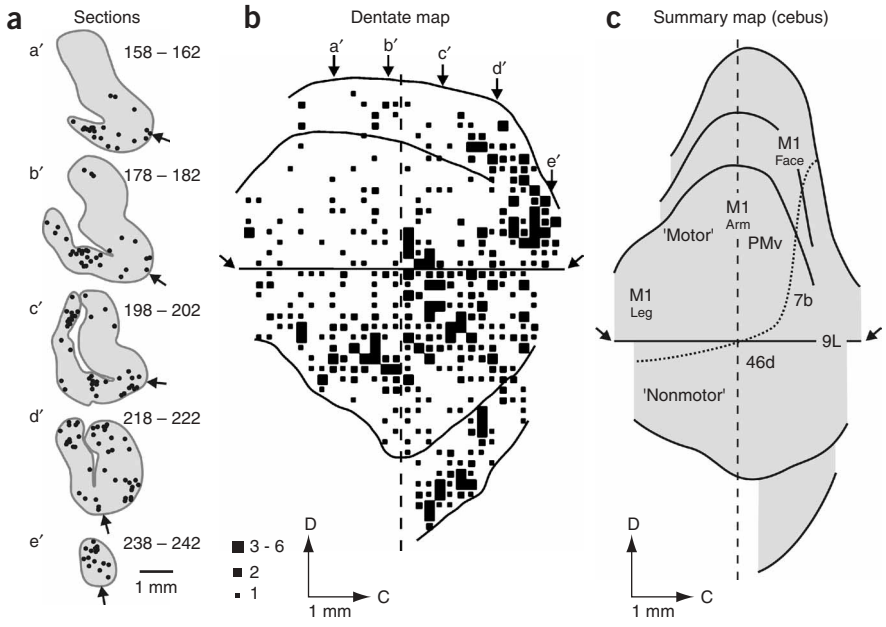

Figure 3 Location of dentate neurons that project to GPe. (**a**) Cross-sections of the dentate. Dots show the location of third-order neurons labeled by retrograde transneuronal transport of virus from GPe. (**b**) Distribution of labeled neurons on an unfolded map of the dentate (for details of map construction, see ref. 10). Arrows at the top of the map in **b** indicate locations of slices in **a**. Arrows in **a** indicate the level of the horizontal line through the middle of the map in **b**. The vertical dashed line marks the rostrocaudal center of the nucleus. Filled squares indicate the density of labeled neurons found in 200 μm × 200 μm bins through the nucleus. (**c**) Motor and nonmotor domains of the dentate (modified from ref. 10). This map shows the origin of dentate projections to different cortical areas ('M1 face', 'M1 arm' and 'M1 leg': face, arm and leg representations in primary motor cortex.; PMv: ventral premotor area; 7b: area 7b in posterior parietal cortex; 9L and 46d: lateral area 9 and dorsal area 46 in prefrontal cortex). D, dorsal; C, caudal. The curved dotted line indicates the border between motor and nonmotor domains of the dentate (for details, see ref. 10).

These observations provide evidence that the output of the dentate is linked to the striatum via a disynaptic connection and to GPe via a trisynaptic connection (**Supplementary Fig. 3**, **Supplementary Discussion**). It is likely that these connections are mediated by intralaminar nuclei and/or ventroanterior/ventrolateral thalamus[6]. The cerebellar nuclei are known to project to these thalamic regions[6], and there is evidence that these thalamic nuclei (especially intralaminar nuclei) project to the striatum[12]. Our findings support a previous study[13] that demonstrated a disynaptic connection between the rat cerebellum and the striatum. The present results extend these observations to a nonhuman primate and demonstrate four new findings: (i) the disynaptic projection to the striatum originates from both the motor and nonmotor domains of the dentate, (ii) the striatum also receives less substantial inputs from fastigial and interpositus, (iii) the projection from the dentate to the striatum connects with medium spiny stellate cells that innervate GPe and thus influences the so-called 'indirect' pathway of basal ganglia processing[14] and (iv) the number of dentate neurons that influence localized portions of GPe is comparable to the number of dentate neurons that influence some areas of posterior parietal and prefrontal cortex[9–11].

The demonstration of a pathway that links the output stage of cerebellar processing to the input stage of basal ganglia processing has broad functional implications. For example, it raises the possibility that the cerebellum adaptively adjusts basal ganglia activity on the basis of some internal model and error signal, in a manner similar to the cerebellar mechanisms for adjusting voluntary movement[15]. Our findings also lead to questions about cerebellar input associated with the motor and cognitive disorders that are characteristic of basal ganglia dysfunction. When basal ganglia activity is abnormal, is cerebellar input part of the problem or part of the solution?

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

This work was supported by the Veterans Affairs Medical Research Service; the National Parkinson Foundation; US Department of Health and Human Services grants MH56661, NS047126, RR018604 (P.L.S.) and a long-term fellowship from the Human Frontier Science Program Organization (E.H.). We thank B. Dietzschold and M. Schnell (Thomas Jefferson University, Philadelphia) for supplying rabies virus and A. Wandeler (Animal Disease Research Institute, Nepean, Ontario, Canada) for supplying antibodies to rabies. We thank M. Page for developing the computer programs; C. Lovell, K. McDonald, M. O'Malley, M. Ratajeski and M. Watach for their technical assistance; and D. Akkal, F. Delis, R.P. Dum, D. Hoffman, and J.-A. Rathelot for scientific discussions.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureneuroscience/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Alexander, G.E., DeLong, M.R. & Strick, P.L. *Annu. Rev. Neurosci.* **9**, 357–381 (1986).
2. Graybiel, A.M. *Neurobiol. Learn. Mem.* **70**, 119–136 (1998).
3. Masterman, D.L. & Cummings, J.L.J. *Psychopharmacology* **11**, 107–114 (1997).
4. Middleton, F.A. & Strick, P.L. *Brain Res. Brain Res. Rev.* **31**, 236–250 (2000).
5. Schmahmann, J.D. *The Cerebellum and Cognition* (Academic, San Diego, 1997).
6. Jones, E.G. *The Thalamus* (Plenum, New York, 1985).
7. Percheron, G., Francois, C., Talbi, B., Yelnik, J. & Fenelon, G. *Brain Res. Brain Res. Rev.* **22**, 93–181 (1996).
8. Kelly, R.M. & Strick, P.L. *J. Neurosci. Methods* **103**, 63–71 (2000).
9. Middleton, F.A. & Strick, P.L. *J. Neurosci.* **21**, 700–712 (2001).
10. Dum, R.P., Li, C. & Strick, P.L. *Ann. NY Acad. Sci.* **978**, 289–301 (2002).
11. Clower, D.M., Dum, R.P. & Strick, P.L. *Cereb. Cortex* **15**, 913–920 (2005).
12. Smith, Y., Raju, D.V., Pare, J.F. & Sidibe, M. *Trends Neurosci.* **27**, 520–527 (2004).
13. Ichinohe, N., Mori, F. & Shoumura, K. *Brain Res.* **880**, 191–197 (2000).
14. Wichmann, T. & DeLong, M.R. *Curr. Opin. Neurobiol.* **6**, 751–758 (1996).
15. Doya, K. *Curr. Opin. Neurobiol.* **10**, 732–739 (2000).