Clarke's Column Neurons as the Focus of a Corticospinal Corollary Circuit

Supplementary Information

Adam W. Hantman and Thomas M. Jessell
Supplementary Figure 1 | Distribution of corticospinal terminals on dSC neurons.

Confocal z-stacks of Clarke’s column from p10–15 thoracic segments of mouse spinal cord. Green, Emx1::GFP+ or Emx1::Cre, Tau::lsl. mGFP+ terminals. Red, GDNF::LacZ+ or CTB-labeled dSC neurons. Blue, VG1. Corticospinal terminals are densest on the dorsomedial boundary of Clarke’s column and sparse on the ventrolateral boundary.
Supplementary Figure 2. Proprioceptive nature of hindlimb afferents in Clarke's column. (a) Parv and VG1 status of hindlimb (HL) CTB-labeled terminals in caudal thoracic Clarke's column of Parv::Cre, Tau::Isl. mGFP mice. Scale bar, 10μm. (b,d,f) Magnified images of boxed area in a. (c,e,g) Parv and VG2 status of hindlimb CTB-labeled afferents in Clarke's column of Parv::Cre, Tau::Isl. mGFP mice. (h) Hindlimb CTB-labeled terminals in lamina II and (i) lamina IV of lumbar level spinal cord.
Supplementary Figure 3  Summary of dSC input from dorsal column axons.
Dorsal column axons with relevance to dSC input include those originating from hindlimb proprioceptors and the cortex. Inhibitory neurons presynaptic to dSC neurons can be stimulated by hindlimb proprioceptors and corticospinal neurons. Forelimb sensory, hindlimb non-proprioceptors, and PSDC neurons do not provide input to dSC neurons.
Supplementary Figure 4: Comparison of forelimb and hindlimb sensory inputs to Clarke’s column.

(a,b) Labeling in spinal cord following forelimb (FL) injection of CTB in p8–10 Parv::Cre, Tau::Lsl. mGFP mice. Scale bar, 50mm.
(d,e) Labeling in spinal cord following hindlimb (HL) injection of CTB in p8–10 Parv::Cre, Tau::Lsl. mGFP mice. Panels 1 and 6 of a,b,d,e are low-magnification images of a hemicord, panels 2–5 are high-magnification images of Clarke’s column. b and d are the CTB signal alone from a and e respectively. (c) Summary of relationship of dSC neurons (blue circles) and the terminals (red) of forelimb (left) and hindlimb (right) sensory afferents. Boxes in c indicate segmental sources of panels 1–6. Approximate segmental levels indicated right of spinal cord.
Supplementary Figure 5  Comparison of forelimb and hindlimb sensory inputs to the brainstem.  (a) Hindlimb CTB-labeled afferent terminals in coronal sections of the brainstems of Parv.Cre, Tau.:Isl. mGFP mice.  Scale bar, 50μm.  (b) Magnified image of hindlimb CTB-labeled afferents in the gracile nucleus.  (c) Forelimb CTB-labeled afferent terminals in coronal sections of the brainstems of Parv.Cre, Tau.:Isl. mGFP mice.  (d) Magnified image of forelimb CTB-labeled afferents in the cuneate nucleus.  (e) Magnified image of forelimb CTB-labeled afferents in the external cuneate nucleus.  (f) Summary of proprioceptive and non-proprioceptive inputs to gracile, cuneate, and external cuneate nuclei.
Supplementary Figure 6. dSC neurons as integrators in a corollary circuit.

(a) Schematic model indicating the distinct organization of cortical and inhibitory inputs to dSC neurons and implications for the relay of information to the cerebellum. (i) dSC neurons that receive peripherally-triggered proprioceptive input (blue) transmit this information to the cerebellum at a time significantly delayed with respect to the onset of a cortical motor command (0). (ii) Descending cortical excitatory input (green) to a subset of dSC neurons permits rapid transfer of information to the cerebellum prior and in addition to peripherally-evoked proprioceptive sensory feedback. (iii) Descending cortical pathways activate inhibitory interneurons that suppress the activity of dSC neurons as well as their proprioceptive input. If predictive inhibitory input is matched to the strength and timing of proprioceptive feedback, sensory afferent input will be cancelled. (iv) Synchronous recruitment of excitatory and inhibitory corollary circuits permits an initial phase of signal transmission, followed by a period in which dSC output is suppressed.

(b) Schematic diagram showing pontocerebellar (*), intracortical (**), and intraspinal (***), corollary discharge pathways. In the spinal cord, the dSC-based corollary circuit outlined here is paralleled by a ventral spinocerebellar transmission system (not shown) which is thought to convey an efference copy of the integrated output of the spinal motor system.
Supplementary Results

Characterizing the origin of primary sensory inputs to Clarke’s column neurons

We used anatomical and genetic methods to define the origin of sensory terminals on Clarke's column dSC neurons.

Different classes of primary sensory neurons in the dorsal root ganglia express the vesicular glutamate transporters VG1 and VG2. Proprioceptors and low threshold mechanoreceptors express VG1, whereas high threshold presumed nociceptive afferents express VG2. In addition, all local excitatory spinal interneurons express VG2. We therefore examined the status of VG1/2 expression in identified sensory terminals on Clarke’s column neurons. To provide a general label of sensory projections to Clarke’s column we injected the hindlimbs of P5–7 mice with cholera toxin B (CTB) subunit and analyzed the synaptic status of CTB-labeled boutons in contact with fluorogold-labeled Clarke’s column neurons 72h later. To label proprioceptive sensory terminals selectively, we examined GFP expression in terminal contacts on Clarke’s column neurons in Parv::Cre, Tau::lsl. mGFP mice.

In Parv::Cre, Tau::lsl. mGFP mice examined after P10 we found that >95% of CTB-labeled terminals in contact with fluorogold-labeled Clarke’s column neurons co-expressed GFP and VG1 and none of them expressed VG2 (Supplementary Fig. 2a-e). Furthermore, >95% of VG1+ sensory terminals in Clarke’s column co-expressed GFP, independent of CTB-labeling status (Supplementary Fig. 2f,g).

Together these findings suggest that proprioceptive afferents provide the predominant, and probably the sole, sensory synaptic input to Clarke’s column dSC neurons.

Stimulation of the ventral aspect of the cervical dorsal column selectively activates corticospinal axons.

In experiments reported in this study, stimulation of the ventral aspect of the dorsal column has been equated with activation of the descending axons of corticospinal neurons. Below, we document several lines of evidence that support this conclusion and, in particular,
argue that responses of Clarke’s column dSC neurons observed after dorsal column stimulation do not reflect the activation of descending or ascending sensory or spinal projection neurons that extend axons into the dorsal columns.

*Differential positioning of corticospinal, sensory, and spinal axons within the dorsal columns:*

The ventral aspect of the dorsal column is comprised almost exclusively of corticospinal axons (Fig. 1a; Supplementary Fig. 1) with axons of sensory or spinal origin restricted to more dorsal tracts within the dorsal columns (shown schematically in Supplementary Fig. 3).

We found that placement of stimulating electrodes on the dorsal aspect of cervical level dorsal column failed to evoke monosynaptic excitatory input to thoraco-lumbar Clarke’s column dSC neurons, whereas more ventral dorsal column stimulation in the same preparation elicited a high incidence of monosynaptic excitatory input (data not shown). This finding prompted us to examine in detail the issue of whether concentric bipolar electrodes placed in the ventral aspect of the dorsal columns activate corticospinal axons in a preferential manner.

*Monosynaptic input to dSC neurons following dorsal column stimulation does not result from orthodromic activation of descending sensory axons.*

We first considered the possibility that cervical dorsal column stimulation might activate the descending branches of primary sensory axons, and that these axons provide monosynaptic input to dSC neurons. Several lines of evidence argue against this possibility.

First, anatomical tracing studies show that sensory axons located in cervical level dorsal columns do not project as far caudally as the thoraco-lumbar Clarke’s column dSC neurons we examined physiologically. To evaluate this, CTB was injected into p7 mouse forelimb, and in separate experiments, sensory axons within cervical dorsal roots were labeled with rhodamine-dextran (Rh-Dex). CTB-labeled sensory terminals were found at low density at rostral thoracic (T1–T6) levels of the spinal cord, and were absent from spinal levels caudal to T6 (Supplementary Fig. 4a-c). Moreover, most of the CTB-labeled terminals found at T4–T6 levels appear not to derive from proprioceptors, since few if any CTB-labeled sensory terminals at T4–T6 spinal levels expressed GFP, examined in *Parv::Cre, Tau::lsl. mGFP* mice (Supplementary
Fig. 4a-3). Similarly, few if any Rh-Dex labeled sensory axons and terminals were detected in the vicinity of Clarke’s column at T6–L2 levels of the spinal cord (data not shown). As controls, we found that CTB-labeled sensory terminals were found in both the dorsal and ventral horns of cervical spinal cord (Supplementary Fig. 4a,b), indicating that this labeling method effectively fills both proprioceptive and cutaneous afferents.

Based on these findings, we conclude that cervical level sensory afferents do not project to Clarke’s column neurons located at caudal thoracic- and lumbar-levels (Supplementary Fig. 3). It is unlikely therefore that monosynaptic EPSCs recorded from Clarke’s column dSC neurons after cervical level dorsal column stimulation reflect activation of the descending branches of sensory afferents.

Monosynaptic input to dSC neurons following dorsal column stimulation does not result from antidromic activation of ascending sensory axons.

We next asked whether hindlimb proprioceptive axons project as far rostrally as the cervical dorsal column. We were concerned that if this is the case, then cervical dorsal column stimulation could antidromically activate proprioceptive axons with collaterals that provide direct input to thoraco-lumbar Clarke’s column dSC neurons.

We mapped the ascending projections of sensory axons that provide input to Clarke's column by injecting CTB into the hindlimb. CTB-labeled sensory terminals were found at high density in lumbar spinal cord, in both dorsal and ventral gray matter (Supplementary Figs. 2h,i; 4d,e), but at progressively decreasing density at more rostral segmental levels, between T12 and T6. Very few CTB-labeled terminals were found rostral to segment T6 (Supplementary Fig. 4c-e). We also monitored the status of GFP expression in the CTB-labeled sensory terminals of Parv::Cre, Tau::lsl. mGFP mice that had received hindlimb CTB injections. CTB⁺, GFP⁺ terminals were not detected in the gray matter of the cervical spinal cord (Supplementary Fig. 4c-e). Together, these findings indicate that hindlimb proprioceptive axons do not give rise to collaterals that enter the cervical gray matter.
We also considered whether hindlimb proprioceptive axons pass through the cervical dorsal columns en route to the brainstem, without sending collaterals into the cervical spinal cord. We therefore examined the origin of sensory axons that terminate in the cuneate, external cuneate, and the gracile nuclei. Consistent with prior studies, hindlimb-derived CTB-labeled terminals were found exclusively in the gracile nucleus (Supplementary Fig. 5a,b,f), whereas forelimb-derived CTB terminals were found in the cuneate and external cuneate nuclei (Supplementary Fig. 5c-f). Importantly, in Parv::Cre, Tau::Isl. mGFP mice, few if any hindlimb injection derived CTB-labeled terminals in the gracile nucleus expressed GFP (Supplementary Fig. 5a,b,f), indicating that these terminals derived from sensory neurons other than proprioceptors. The lack of GFP labeling was not the consequence of inadequate GFP expression at the more rostral brainstem sites, since in Parv::Cre, Tau::Isl. mGFP mice, many forelimb-derived CTB-labeled terminals in the external cuneate nucleus did express GFP (Supplementary Fig. 5c,e,f). We conclude that proprioceptive sensory axons do not project to brainstem termination zones nor do they form a terminal projection zone at cervical levels of the spinal cord. Thus, cervical level dorsal column stimulation does not result in antidromic activation of ascending proprioceptive axons (see Supplementary Fig. 3 for schematic diagram). Our data showing that hindlimb proprioceptive inputs do not ascend into the cervical spinal cord in the mouse is in agreement with physiological and anatomical studies of ascending proprioceptive pathways in other species.

Cervical dorsal column stimulation does not antidromically activate sensory axons which activate inhibitory inputs to dSC neurons:

The findings described above leave unresolved the issue of whether cervical dorsal column stimulation could antidromically activate the axons of non-proprioceptive sensory afferents of hindlimb origin. These sensory axons could, in principle, send caudal collaterals onto inhibitory interneurons that project to Clarke’s column dSC neurons. In this view, inhibitory responses in dSC neurons recorded after cervical dorsal column stimulation could reflect antidromic sensory rather than orthodromic corticospinal axonal activation.

Against this idea, there is extensive evidence that activation of cutaneous sensory inputs does not activate disynaptic inhibitory pathways that project to Clarke's column. Instead,
cutaneous stimulation appears to facilitate muscle afferent-evoked inhibition of dSC neurons, likely by dis-inhibiting muscle afferents themselves\textsuperscript{52}. The fact that peripherally-evoked cutaneous input fails to recruit inhibitory neurons pre-synaptic to Clarke's column neurons and their location in the dorsal aspects of the dorsal column makes it unlikely that antidromic stimulation of cutaneous sensory axons underlies the dorsal column-evoked inhibition of dSC neurons observed in our studies (Supplementary Fig. 3).

\textit{Dorsal column stimulation does not antidromically activate the ascending axons of post-synaptic dorsal column neurons which, in turn, activate inhibitory inputs to dSC neurons:}

The dorsal aspect of the dorsal columns also contains the axons of post-synaptic dorsal column (PSDC) pathway neurons\textsuperscript{44}. The main source of sensory input to PSDC neurons is from cutaneous afferents\textsuperscript{44}. But activation of cutaneous afferents is ineffective in inhibiting dSC neurons and so, adhering to the logic of the preceding section, it is unlikely that antidromic activation of the axons of PSDC neurons by cervical level dorsal column stimulation will recruit inhibitory inputs to Clarke's column (see Supplementary Fig. 3 for a schematic of PSDC pathways).

From this series of control experiments, we conclude that under our focal stimulation conditions, stimulation of the ventral aspect of cervical dorsal columns results in selective activation of the axons of corticospinal projection neurons.
Supplementary Notes

42. Todd, A. J. et al. The expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in neurochemically defined axonal populations in the rat spinal cord with emphasis on the dorsal horn. Eur J Neurosci 17, 13-27 (2003)

43. Persson, S. et al. Distribution of vesicular glutamate transporters 1 and 2 in the rat spinal cord, with a note on the spinocervical tract. J Comp Neurol 497, 683-701 (2006)

44. Brown, A. G. & Fyffe, R. E. Form and function of dorsal horn neurones with axons ascending the dorsal columns in cat. J Physiol 321, 31-47 (1981)

45. Brown, A. G. Organization in the spinal cord: the anatomy and physiology of identified neurones. 238 (Springer-Verlag, 1981).

46. Maslany, S., Crockett, D. P. & Egger, M. D. Somatotopic organization of the dorsal column nuclei in the rat: transganglionic labelling with B-HRP and WGA-HRP. Brain Res 564, 56-65 (1991)

47. Lloyd, D. P. & Mc, I. A. Dorsal column conduction of group I muscle efferent impulses and their relay through Clarke's column. J Neurophysiol 13, 39-54 (1950)

48. Perl, E. R., Whitlock, D. G. & Gentry, J. R. Cutaneous projection to second-order neurons of the dorsal column system. J Neurophysiol 25, 337-358 (1962)

49. Hongo, T. et al. Inhibition of dorsal spinocerebellar tract cells by interneurones in upper and lower lumbar segments in the cat. J Physiol 342, 145-159 (1983)

50. Hongo, T. et al. The same interneurones mediate inhibition of dorsal spinocerebellar tract cells and lumbar motoneurones in the cat. J Physiol 342, 161-180 (1983)

51. Jankowska, E. & Puczynska, A. Interneuronal activity in reflex pathways from group II muscle afferents is monitored by dorsal spinocerebellar tract neurons in the cat. J Neurosci 28, 3615-3622 (2008)

52. Jimenez, I., Rudomin, P. & Solodkin, M. PAD patterns of physiologically identified afferent fibres from the medial gastrocnemius muscle. Exp Brain Res 71, 643-657 (1988)