Supplemental Material

Supplemental Figure S1. Representative example of unilateral PT lesion. The lesion was made using a wire knife (1.1 mm; David Kopf Instruments, CA) deployed at a depth of 1.1 mm below the surface adjacent to the basilar artery and gently lifted to lesion all PT axons within the wire knife arc. A. The dashed line marks the right pyramid rostral to the level of the lesion. Note, axons in the left PT are densely labeled with BDA after injections into motor cortex. B. This is a section through the lesion site. The left PT is completely lesioned. The right PT is spared. Calibration: 200 μm.

Supplemental Figure S2. Representative recording from the contralateral and ipsilateral deep radial nerves in response to a PT stimulus train in a stimulation only animal. Each trace is an average (n=30 trials) electroneurogram (ENG) in response to 3 stimulus pulses. The stimulus artifacts are blanked and replaced by the gray rectangles. A1. Evoked ENG response in the contralateral deep radial nerve at threshold (112μA). B1. Ipsilateral response at the threshold for evoking the contralateral response. A2. Contralateral response at threshold for evoking the ipsilateral response (B2, 175μA). Calibration bar: A1. 10 μV; A2. 30 μV; B1. 100 μV; B2. 30 μV;

Supplemental Material 3. Experiments to verify selective CST activation with surface PT stimulation.

Our approach to activating the CST using an electrode mounted on the ventral pyramid surface was selective, based on two control experiments. First, lesion of the stimulated pyramid abolished the DRN response (Supplemental Figure S3 A,B). Second, subsequent stimulation at higher currents, or from within the reticular formation dorsal to the pyramid, evoked responses that were substantially shorter than the responses evoked in the four animal groups (Supplemental Figure S3C).

Our goal was to selectively stimulate the CST. At higher currents, PT stimulation might activate other descending motor systems, the most likely being the reticulospinal tract, as the reticular formation lies dorsal to the PT. We tried to distinguish the specificity of the stimulation based on the latency of the response. Various studies show that the reticulospinal tract has a substantially faster conduction velocity than the CS tract(and thicker myelinated axons), suggesting that responses evoked by the reticulospinal tract have shorter latencies than those evoked by the CS tract (Peterson et al., 1975; Stewart et al., 1990; Kawai and Nagao, 1992; Brosamle and Schwab, 2000) Casale, 1991 #4487}. To test this idea, we first conducted acute experiments (n=3 rats) to determine if short latency DRN responses could be evoked dorsal to the pyramid, consistent with a shorter latency non-pyramidal pathway. We found that surface

stimulation, at threshold, evoked responses at latencies that were consistently longer than responses evoked from within the ventral medulla 1.2 mm below the surface (~14 ms v/s 8-10 ms). We next compared DRN responses before and after lesion of the PT to eliminate CS tract-evoked DRN responses. Stimulation of the intact PT with the surface electrode evoked contralateral and ipsilateral responses at the same latencies (~14 ms; Figure 2A) that were subsequently eliminated by PT lesion (Figure 2B). Stimulation rostral to the PT lesion at suprathreshold stimulation (4x the contralateral response; 450 μA) evoked bilateral responses at short latency (~9-10 ms; Figure 2C). Our finding that threshold stimulation after PT lesion evoked bilateral non-pyramidal responses at substantially shorter latencies (and at higher currents) is consistent with reticulospinal activation.

We next determined if the latency of ipsilateral responses (evoked at threshold) were different from the threshold contralateral response. Across the entire dataset, there was no significant difference (paired t-test; P =0.183). Moreover, ipsilateral response latency did not differ across groups (ANOVA; p=0.505), nor was there a correlation between the current to evoke an ipsilateral DRN response and the latency of evoked response (R=0.101; NS). Similarly, contralateral latency did not vary across groups (ANOVA; p=0.298), nor were current and contralateral latency related (R=0.229; NS).Taken together, the results of the acute control experiments and our analyses of the latencies of DRN responses in the different experimental animal groups strongly suggest that the ipsilateral and contralateral responses are both likely to have been evoked by activation of the CS tract axons in the PT.

Supplemental Figure S3. PT surface stimulation selectively activates the CST. A. Ensemble averages (n=30 trials) of ENG responses to threshold stimulation of the intact PT (contralateral threshold = 120μA; ipsilateral threshold = 260μA). B. As A, but following acute bilateral transection of the PT. No responses were seen at stimulus current just above ipsilateral threshold (300μA). C. As B, but after further increasing current to nearly four times the contralateral threshold (450μA). This stimulus evoked a bilateral response with markedly shorter onset latency than responses evoked from the intact PT. This is consistent with reticulospinal tract activation. Calibrations: A,B,C contral 10 μV; A,B,C ipsi 5 μV.