

Supporting Information

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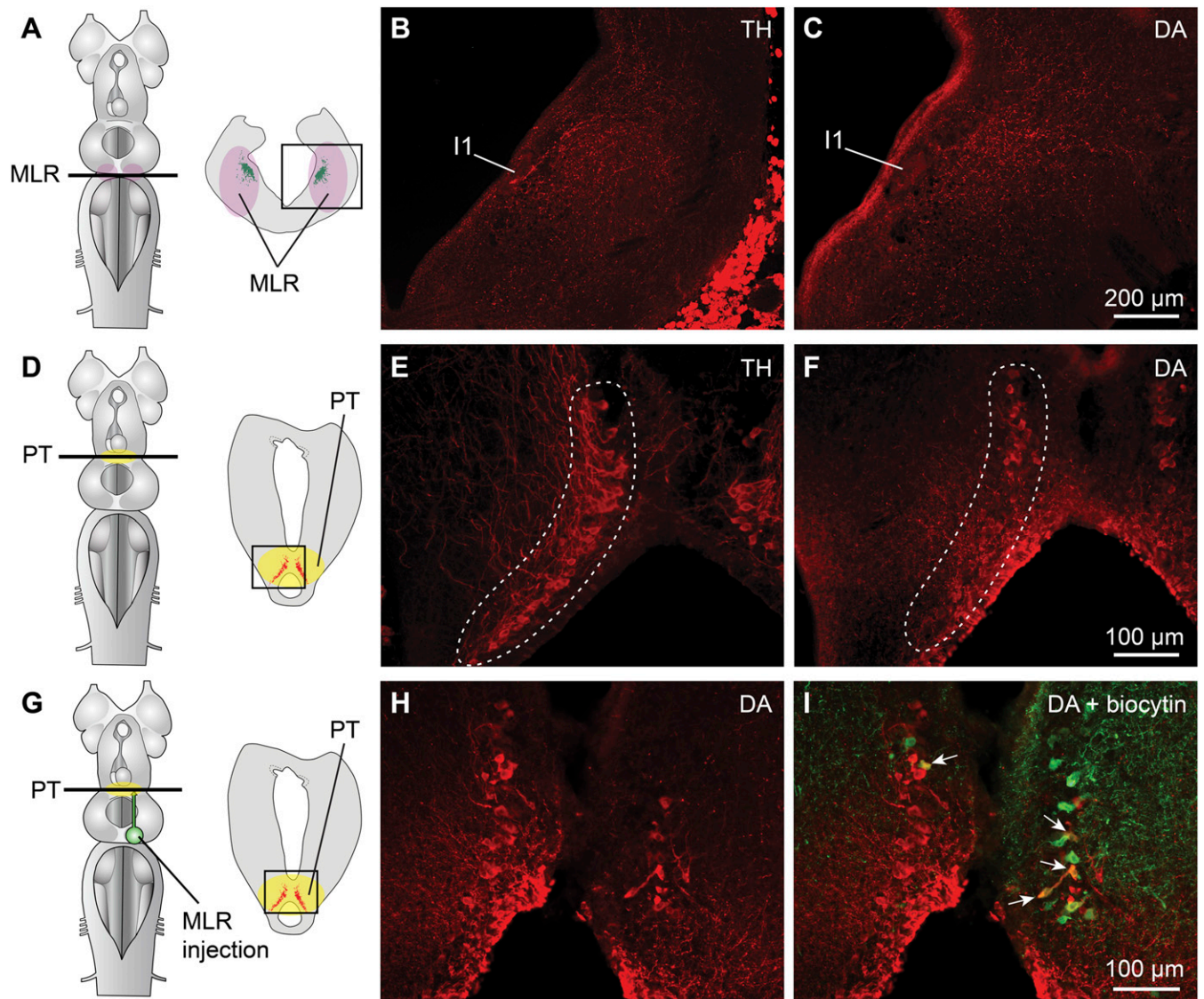


Fig. S1. Comparison of tyrosine hydroxylase (TH) and dopamine (DA) immunofluorescence distribution in the mesencephalic locomotor region (MLR) and posterior tuberculum (PT). TH and DA results were obtained from different animals as a result of the different fixation methods. (A–C) Photomicrographs showing that the distribution and number of TH and DA immunoreactive fibers and varicosities are similar in the MLR. The giant isthmic reticulospinal neuron I1 is indicated as a landmark. (D–F) The distribution of TH and DA immunoreactive neurons in the PT showed a high degree of similarity in the outlined area, where neurons were found to project to the MLR and striatum. Neurons appear less numerous under DA immunofluorescence as a result of the glutaraldehyde fixation that prevents deep penetration of antibodies into 25- μ m sections. (G and I) Unilateral injection of the tracer biocytin in the MLR (green) coupled with immunofluorescence against DA (red, H and I) revealed many double-labeled cells in the PT (white arrows in I), as it is the case when using TH immunofluorescence (Fig. 1).

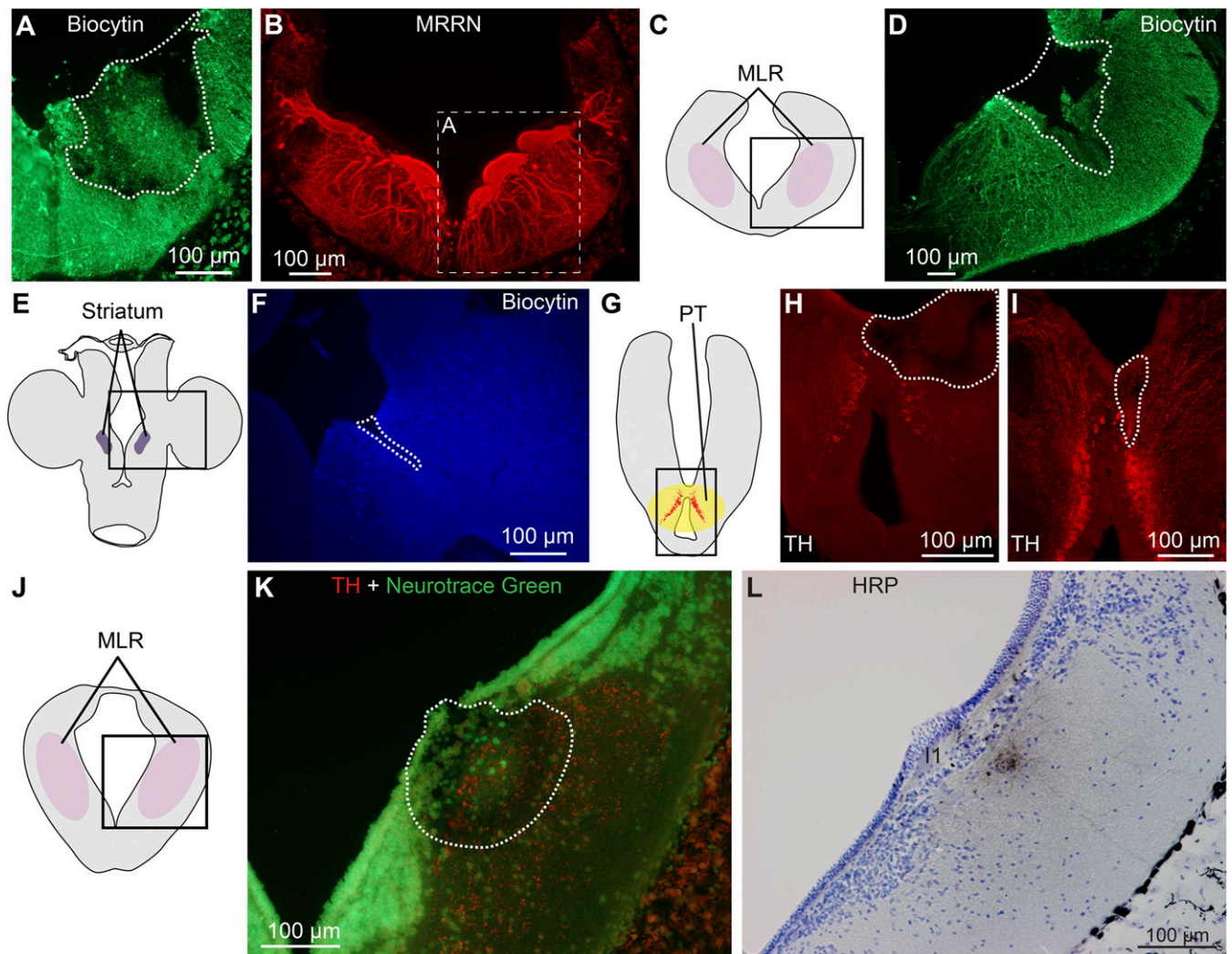


Fig. S2. Histological controls for stimulation, recording, and injection sites. (A) Typical unilateral injection site (enclosed by white dashed line) of the retrograde tracer biocytin at the level of the middle rhombencephalic reticular nucleus (MRRN), aiming at the cell bodies and extensive dendrite trees of these reticulospinal neurons (B). (B) In parallel, a preparation was injected in the first spinal segments to label the reticulospinal neurons of the middle rhombencephalic reticular nucleus with their dendritic trees. The white rectangle area corresponds to the photographed region in A. (C and D) Typical unilateral tracer injection site at the level of the MLR used to label neurons in the PT. The injection site delineated in D (enclosed by white dashed line) coincides with the caudal MLR cholinergic neuronal population of the laterodorsal tegmental nucleus (schematized in C) and with a large number of the dopaminergic fibers shown in Fig. S1 B and C. A large part of the labeling that is seen around the injected area consists of local radial glial cells. (E and F) Typical unilateral tracer injection site (enclosed by white dashed line, F) in the striatum used to retrogradely label neurons in the PT. (G–I) Stimulation sites in the PT were confirmed by electrolytic lesions that coincided with the location of TH-positive neurons (red) in preparations used for patch-clamp recordings (H) or for voltammetry experiments (I). The same controls were also carried out for semi-intact preparations (Fig. 3D). The extent of the electrolytic lesion is delineated by the white dashed regions in H and I. (J and K) The electrolytic lesion performed in the MLR with the voltammetry electrode is delineated by the area enclosed by the white dashed line in K. The location where DA release was detected coincides with the location of TH-positive fibers (red) in the MLR. NeuroTrace green was used as a Nissl counterstain. (L) Histological control of the extent a microinjection of a HRP solution [10% (wt/vol)] in the MLR region using a Picospritzer. The HRP solution was injected in four larval lampreys following the same parameters used to inject DA compounds in the MLR as well as D-glutamate in the PT. Approximately 30 s after the last injection, the preparation was rapidly fixed and processed histologically to evaluate the spread of those injections. Here HRP was revealed by using 3,3'-diaminobenzidine over a cresyl violet Nissl counterstain. Throughout this study, biocytin was revealed in different colors from one experiment to another.

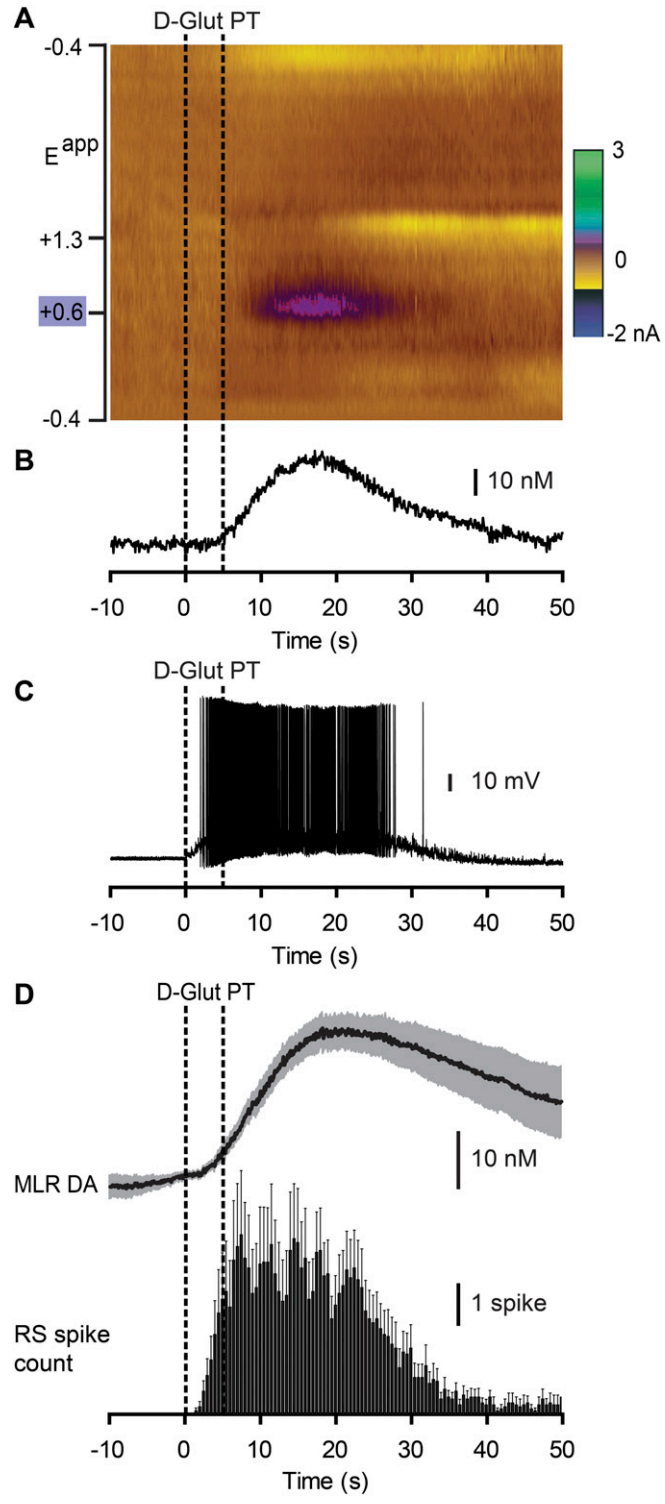


Fig. S3. Local activation of neuronal cell bodies of the PT by microinjections (5-s train, 2 Hz, 100-ms pulses) of D-glutamate (5 mM; *Materials and Methods*) evokes DA release in the MLR and concurrent reticulospinal (RS) activity. (**A**) DA release evoked by D-glutamate microinjection in the PT (D-Glut PT) in an isolated brain preparation (stimulation period = 0–5 s). The color plot depicts current changes (in color) across the applied voltages (E^{app} ; i.e., ordinate) over time (i.e., abscissa). DA is identified by its oxidation peak (~ 0.6 V) that appears during PT stimulation. As is the case when stimulating the PT electrically (Fig. 4), here the DA electrochemical signal (cyclic voltammogram) recorded from the MLR in **A** is similar ($R = 0.97$, $P < 0.001$) to that measured from a 1- μ M control DA solution, thus confirming DA detection. (**B**) Changes in DA concentration in the MLR (extracted from **A**). (**C**) Spiking activity in a reticulospinal cell of the middle rhombencephalic reticular nuclei elicited by D-glutamate microinjection in the PT. (**D**) Changes in DA concentration plotted vs. the number of reticulospinal spikes per unit time (bin represents 500 ms) in the same trials ($n = 20$ microinjections from four preparations) indicates that the DA release in the MLR elicited by D-glutamate microinjection in the PT was positively correlated with the number of spikes evoked in reticulospinal cells ($R = 0.50$, $P < 0.001$). Mean \pm SEM are illustrated. Data from **A** and **B**, and data from **C**, are from two different preparations that were included in **D**.

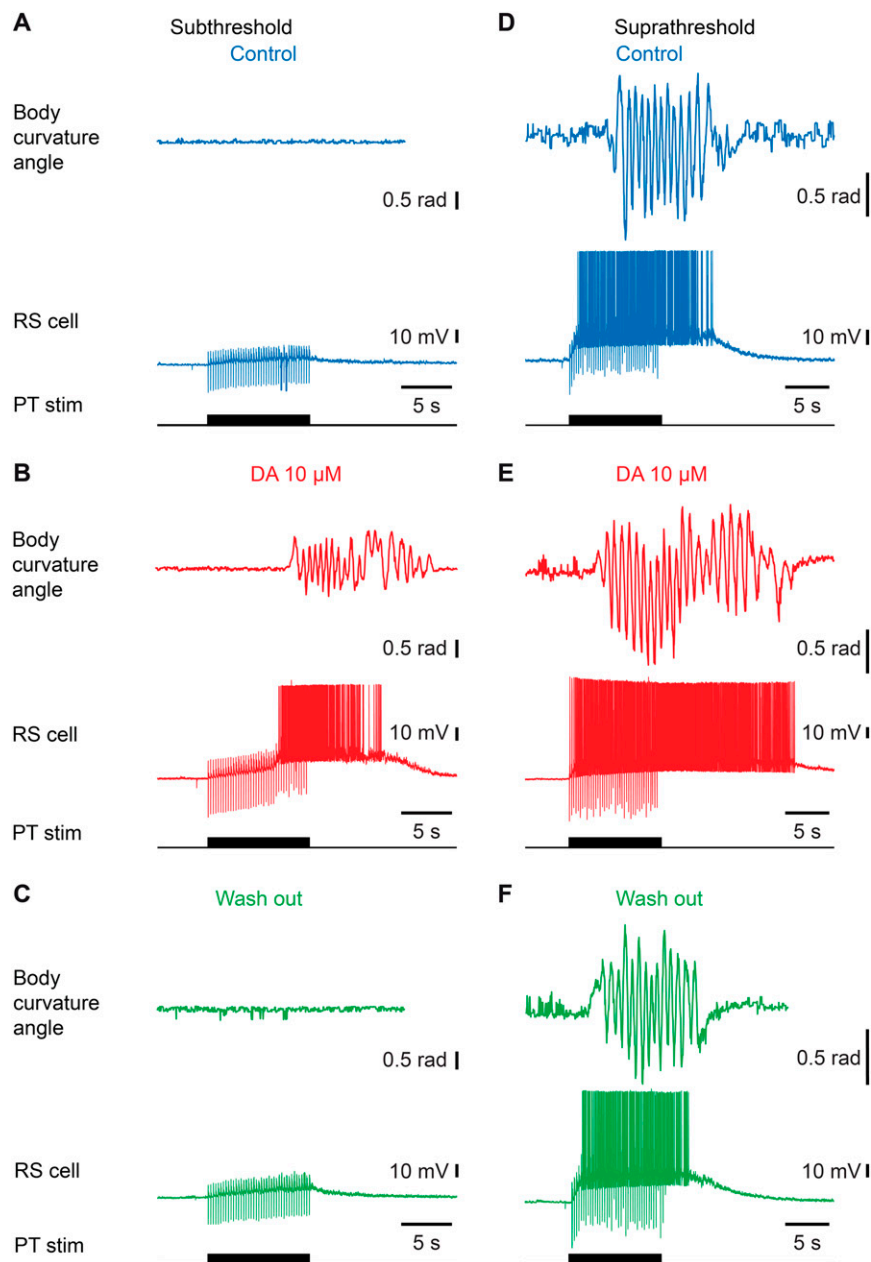


Fig. S4. DA increases the locomotor output elicited by stimulation of the PT in a semi-intact preparation. (A–C) Bath-applied DA (10 μ M) onto the brain (partitioned bath; *Materials and Methods*) decreased the current intensity (for the illustrated animal, reduction from 16 to 12 μ A) required to elicit locomotion when stimulating the PT (here 10-s train, 4 Hz, 12 μ A, 2-ms pulses). (D–F) When stimulation intensity was above the threshold for eliciting swimming, bath-applied DA increased the locomotor output elicited by PT stimulation (here 10-s train, 4 Hz, 26 μ A, 2-ms pulses). In the illustrated animal, DA reversibly increased the range of locomotor bout duration (control, 0.9–11.4 s; injection, 12.1–20.3 s; wash, 2.9–15.5 s), number of locomotor cycles (control, 1–14 cycles; injection, 10–21 cycles; wash, 3–16 cycles), locomotor frequency (control, 0.3–1.3 Hz; injection, 1.0–1.7 Hz; wash, 0.9–1.4 Hz), number of spikes in reticulospinal (RS) neurons (control, 18–288 spikes; injection, 73–561 spikes; wash, 21–202 spikes) and the duration of spiking activity in reticulospinal neurons (control, 4.3–15.4 s; injection, 9.0–24.8 s; wash, 2.4–15.3 s) elicited by a range of PT stimulations (16, 18, 20, 22, 24, and 26 μ A). In all panels, blue illustrates the control condition, red the bath-applied DA condition, and green the washout condition. For each condition, the body curvature oscillations measured during locomotion are illustrated with the corresponding spiking activity of a reticulospinal neuron. Data from A–C and D–F are from two different animals.