Motor, cognitive, and affective areas of the cerebral cortex influence the adrenal medulla

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Modern medicine has generally viewed the concept of "psychosomatic" disease with suspicion. This view arose partly because no neural networks were known for the mind, conceptually associated with the cerebral cortex, to influence autonomic and endocrine systems that control internal organs. Here, we used transneuronal transport of rabies virus to identify the areas of the primate cerebral cortex that communicate through multisynaptic connections with a major sympathetic effector, the adrenal medulla. We demonstrate that two broad networks in the cerebral cortex have access to the adrenal medulla. The larger network includes all of the cortical motor areas in the frontal lobe and portions of somatosensory cortex. A major component of this network originates from the supplementary motor area and the cingulate motor areas on the medial wall of the hemisphere. These cortical areas are involved in all aspects of skeletomotor control from response selection to motor preparation and movement execution. The second, smaller network originates in regions of medial prefrontal cortex, including a major contribution from pregenual and subgenual regions of anterior cingulate cortex. These cortical areas are involved in higher-order aspects of cognition and affect. These results indicate that specific multisynaptic circuits exist to link movement, cognition, and affect to the function of the adrenal medulla. This circuitry may mediate the effects of internal states like chronic stress and depression on organ function and, thus, provide a concrete neural substrate for some psychosomatic illness.

cerebral cortex | sympathetic | psychosomatic | rabies virus

Everyone has experienced an "adrenaline rush," an acute response to stress. Cannon (1) showed a century ago that this psychological experience and its physiological correlates (e.g., increased heart rate, sweating, pupillary dilation) involve secretion from the adrenal medulla triggered by sympathetic neurons in the thoracic spinal cord. He noted that these responses are anticipatory, preparing the body for "fight or flight."

Sympathetic activation is equally essential for precise, organ-specific responses during exercise, exposure to heat or cold, and hypoglycemia, and activation can occur during cognitive deliberations and stressful social situations (2, 3). For example, we all have our own "hot buttons"—issues or events that can trigger an immediate intense reaction. Commonly, the event can be a critical remark from a supervisor, parent, or spouse. The most effective behavior in these circumstances is neither fight nor flight, but rather a more nuanced response that is adjusted to the context of the situation. Clearly, the response to stress is subject to extensive "top-down" or cognitive control (2, 4–6). Here, we used transneuronal transport of rabies virus (RV) to identify the areas of the cerebral cortex that are responsible for the top-down control of the adrenal medulla in a nonhuman primate.

RV is transported exclusively in the retrograde direction and moves transneuronally in a time-dependent manner (7). By varying the survival time, it is possible to trace synaptically linked circuits of up to six neurons in length. In the past, we have used the transneuronal transport of RV to define the location of cortical neurons that control specific muscles (8, 9) (Fig. 1, *Left*). Here, we injected RV (N2c strain) into the adrenal medulla and set the survival time in different animals to allow transport through chains of 2–4 synaptically linked neurons (second-order animal: $n = 1$; third-order animal: $n = 1$; fourth-order animals: $n = 4$) (Fig. 1, *Right*, and Fig. S1). Infected neurons were distributed throughout the brainstem and diencephalon in second- to fourth-order animals in patterns comparable to those described for rodents when similar studies were performed by using pseudorabies virus (10). This report will focus on the distribution of infected neurons in the cerebral cortex.

We first observed substantial numbers of infected neurons (mean = 5,232) in the cerebral cortex in fourth-order animals. Most of these infected neurons were located in layer V, the main source of descending outputs from the cerebral cortex (Fig. S2). Retrograde transneuronal transport of RV from an injection site in the adrenal medulla infected neurons largely (87%) in two nonoverlapping sets of cortical areas: a "motor" network (11–13) and a "medial prefrontal" network (14) (Fig. 2). Small numbers of infected neurons also were located in areas within the lateral sulcus (~7%) and orbitofrontal cortex (~3%).

The motor network was the major source of descending influence over the adrenal medulla (63% of all labeled neurons) (Fig. 2*F*). This network included all seven of the cortical motor areas in the frontal lobe and specific regions of somatosensory cortex (areas 3a, 1, 2) and posterior parietal cortex (area 5) (Figs. 2 and 3). All of the motor areas projected primarily to the contralateral adrenal medulla (contralateral to ipsilateral ratio ~85:15) except for the rostral cingulate motor area (CMAr),

Significance

How does the "mind" (brain) influence the "body" (internal organs)? We identified key areas in the primate cerebral cortex that are linked through multisynaptic connections to the adrenal medulla. The most substantial influence originates from a broad network of motor areas that are involved in all aspects of skeletomotor control from response selection to motor preparation and movement execution. A smaller influence originates from a network in medial prefrontal cortex that is involved in the regulation of cognition and emotion. Thus, cortical areas involved in the control of movement, cognition, and affect are potential sources of central commands to influence sympathetic arousal. These results provide an anatomical basis for psychosomatic illness where mental states can alter organ function.

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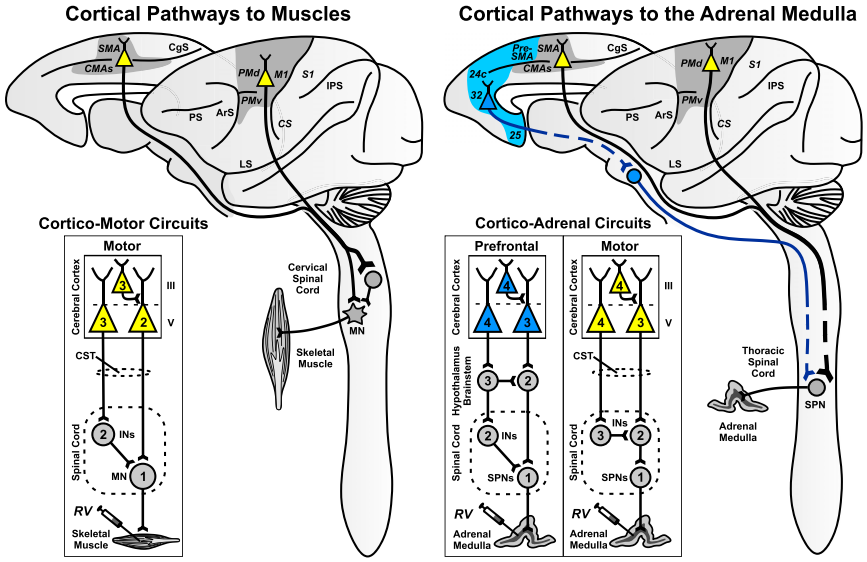

Fig. 1. Mind–body circuits. (*Left*) Cortical pathways to muscles (8, 9). Cortical areas on the lateral surface and the medial wall of the hemisphere are the source of neurons that project to the spinal cord and influence motoneurons that innervate muscles. *Inset* (cortico-motor circuits) is a schematic diagram of the circuits that connect neurons in motor areas of the cerebral cortex to single muscles. When RV is injected into a muscle, it is transported transneuronally in the retrograde direction through these circuits. Depending on the survival time, the virus will infect first-order (1), second-order (2), or third-order (3) neurons. (*Right*) Cortical pathways to the adrenal medulla. Cortical areas on the lateral surface and the medial wall of the hemisphere are the source of neurons that influence the adrenal medulla. *Inset* (cortico-adrenal circuits) is a schematic diagram of the multisynaptic circuits that connect neurons in the motor and prefrontal areas of the cerebral cortex to the adrenal medulla. When RV is injected into the adrenal medulla, it is transported transneuronally in the retrograde direction through these circuits. Depending on the survival time, the virus will infect first-order (1), second-order (2), third-order (3), or fourth-order (4) neurons. Gray shading, cortical motor areas; blue shading, medial prefrontal areas. ArS, arcuate sulcus; CgS, cingulate sulcus; CMAs, cingulate motor areas; CS, central sulcus; CST, corticospinal tract; INs, interneurons; IPS, intraparietal sulcus; LS, lateral sulcus; M1, primary motor cortex; MN, motoneurons; PMd and PMv, dorsal and ventral premotor areas; PS, principal sulcus; S1, primary somatosensory cortex; SMA, supplementary motor area; SPNs, sympathetic preganglionic neurons.

which projected bilaterally. The strongest projections originated from the "trunk" representation of the primary motor cortex (M1), from the dorsal premotor area (PMd) on the lateral surface of the hemisphere, and from the four motor areas on the medial wall of the hemisphere [the supplementary motor area (SMA), and the rostral, dorsal, and ventral cingulate motor areas (CMAr, CMAd, and CMAv)]. Each of the cortical motor areas has a human equivalent. On the medial wall, the CMAr and CMAv of the monkey are considered to be the rostral cingulate zone (RCZ) in humans and the CMAd is the caudal cingulate zone (CCZ) in humans (15, 16) (Fig. 4 *A*–*C*).

The cortical areas that form the motor network are richly interconnected (12, 13). Furthermore, all of these cortical motor areas project directly to the spinal cord (11, 13) and to regions of the reticular formation (17). Thus, descending cortico-spinal and corticobulbo-spinal pathways may mediate some or all of the influence of the motor network on the adrenal medulla (Fig. 5 and Fig. S1). This conclusion is supported by classic studies that demonstrated that surface stimulation in restricted regions of M1 and the PMd evoked changes in blood pressure (18). These changes survived lesions of the fifth nerve and hypothalamus, but were abolished by lesions of the pyramidal tract (18, 19).

The medial prefrontal network represented nearly 25% of the descending cortical output to the adrenal medulla (Fig. 2*F*). This network originated from multiple, distinct regions of medial prefrontal cortex on the medial wall of the hemisphere and on the lateral surface of the hemisphere (Figs. 2 and 3). Ipsilateral projections from the medial prefrontal network to the adrenal medulla were approximately twice as numerous as the contralateral projections. The core of this network originated from two regions of anterior cingulate cortex (ACC)—a "pregenual" region including portions of areas 32 and 24 and a "subgenual" region consisting mainly of area 25 (Figs. 2 and 3). Comparable pregenual and subgenual regions of the ACC have been identified in humans (Fig. 4 *A*–*C*) (20–22).

The pregenual and subgenual regions of the anterior cingulate cortex are densely interconnected (14). These cortical areas in nonhuman primates do not project directly to the spinal cord. Instead, neurons in these regions were probably infected through their reported projections to the medullary reticular formation, hypothalamus, and periaqueductal gray (14, 17, 23), which, in turn, project to brainstem sympathetic nuclei and to the spinal cord (Fig. 1 and Fig. S1) (24–26).

To identify cortical areas that may be less directly connected to the adrenal medulla, we extended the survival time to allow transport through chains of 5–6 synaptically linked neurons (fifth-order animals: $n = 2$; and sixth-order animals: $n = 2$). Prolonging the survival time resulted in a dramatic increase (20- to 100-fold) in the numbers of labeled neurons in the cerebral cortex (fifth order, mean = ~104,000; sixth order, mean = ~480,000). Large numbers of labeled neurons were located not only in layer V, but also in supra- and infragranular layers of cortex. However, the cortical areas with dense labeling (i.e., local peak density, top 15% of bins) in sixth-order animals remained the same as those that were densely labeled in fourth-order animals (Figs. 2 and 3). As with the shorter survival times, smaller concentrations of infected neurons also were located in cortical areas in the lateral sulcus (<11%) and in regions of orbitofrontal cortex (<6%). No other cortical regions contained more than 2% of the labeled neurons. Thus, independent of the survival time, the motor and prefrontal networks are the major sources of descending influence over the adrenal medulla.

Discussion

Our results have a number of important functional implications. First, they emphasize the importance of the cortical motor areas

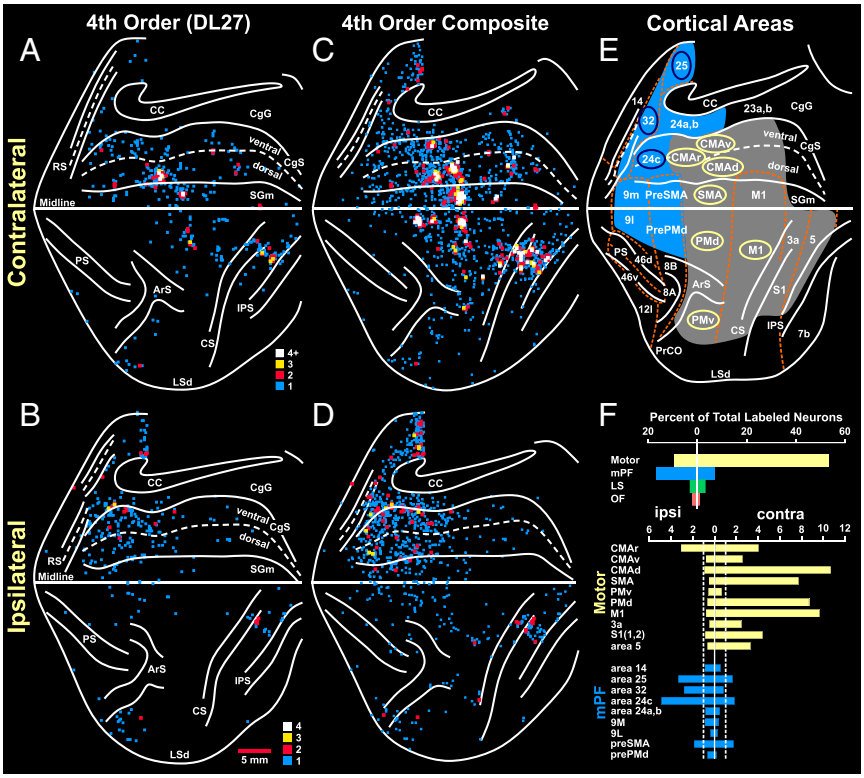

Fig. 2. Cortical origin of multisynaptic inputs to the adrenal medulla. (*A* and *B*) Flat map of the distribution of neurons in the frontal and parietal lobes after RV was injected into the adrenal medulla (case DL27). (*A*) Contralateral hemisphere. (*B*) Ipsilateral hemisphere. The medial wall of the hemisphere is reflected upwards (11). These results were obtained by using a survival time that allowed retrograde transneuronal transport of rabies to label fourth-order neurons. The colored squares indicate the number of labeled neurons found in successive 200-μm bins found on each 50-μm section (color key). (*C* and *D*) Composite maps of the distribution of fourth-order neurons in the frontal and parietal lobes of four animals injected with rabies into the adrenal medulla. (*C*) Contralateral hemisphere. (*D*) Ipsilateral hemisphere. (*E*) Cortical areas under analysis. Motor and somatosensory areas are shaded gray. Medial prefrontal areas are shaded blue. Cortical motor areas in the frontal lobe are indicated by the yellow ellipses; specific areas of cingulate cortex are indicated by blue ellipses. (*F*) Histograms of the distribution of labeled neurons in cortical areas contralateral (right) and ipsilateral (left) to the injection site. Motor areas, yellow bars; medial prefrontal areas, blue bars; lateral sulcus areas (LS), green bar; orbitofrontal areas (OF), red bar. Top histogram, summary distribution; bottom histogram, distribution in specific areas. Dashed lines indicate 1% level of the total number of infected cortical neurons. CC, corpus callosum; CgG, cingulate gyrus; CMAd, CMAr, and CMAv, dorsal, rostral, and ventral cingulate motor areas; LSd, dorsal lip of the lateral sulcus; mPF, medial prefrontal cortex; RS, rostral sulcus; SGm, medial superior frontal gyrus. Other abbreviations defined in the legend of Fig. 1.

in the top-down influence over the adrenal medulla. Normal limb and body movements are accompanied by coordinated changes in sympathetic output that are proportional to the metabolic demands of the task. An important component of this sympathetic activity is thought to be due to a "central command" that can precede any change in muscle activity (4–6). This command is a "predictive" response because it prepares the body to meet the anticipated metabolic needs of activities like exercise, emotional states, and fight or flight. Our results suggest that the central commands to prepare for and generate visceromotor

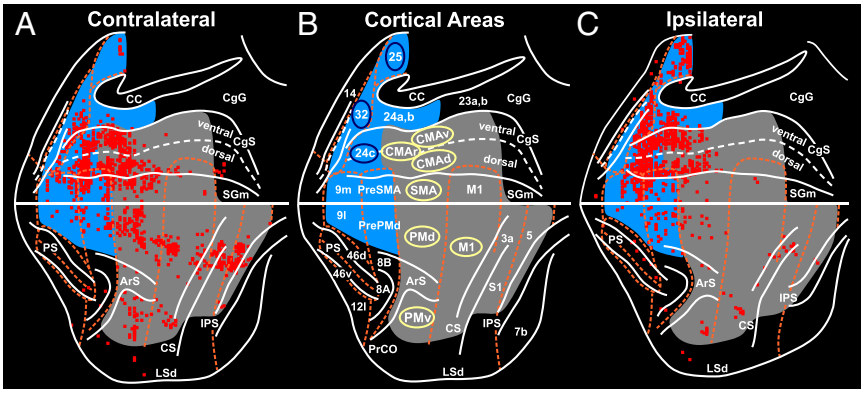

Fig. 3. Densest cortical origin of multisynaptic inputs to the adrenal medulla. The survival time in this animal allowed retrograde transneuronal transport of rabies to label sixth-order neurons. The red squares indicate 200-μm bins on single sections that contained the largest number of labeled neurons (top 15%). (*A*) Contralateral hemisphere. (*C*) Ipsilateral hemisphere. (*B*) Areas of the cerebral cortex in the diagram. Conventions and abbreviations as in Fig. 2.

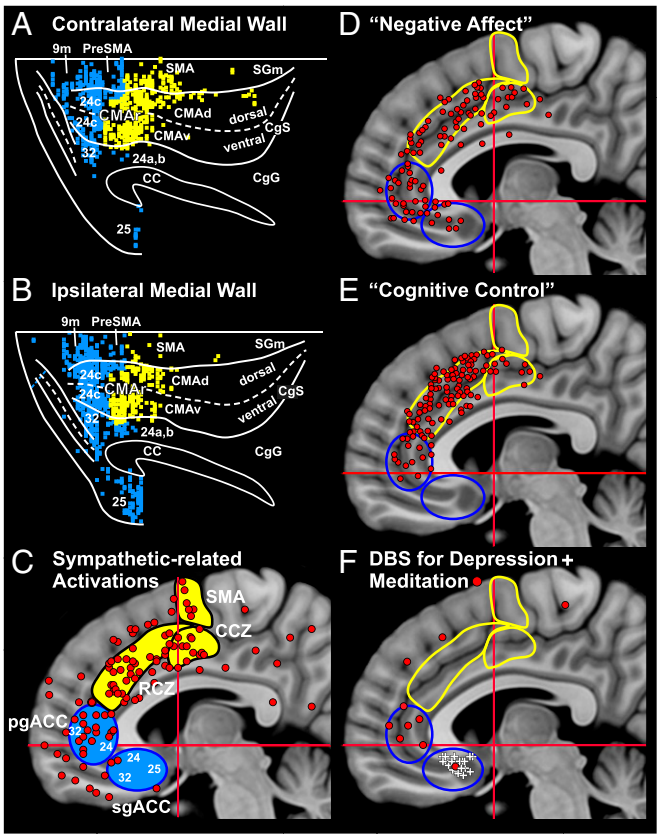

Fig. 4. Monkey anatomy and human neuroimaging: comparison of results. The densest origin of projections to the adrenal medulla are color-coded: motor areas, yellow bins; medial prefrontal areas, blue bins (from Fig. 3). (*A*) Contralateral medial wall of the hemisphere. (*B*) Ipsilateral medial wall. Dorsal is at the top and anterior is to the left for all of the diagrams in this figure. (*C*–*F*) Sites of activation on the medial wall of the hemisphere found in human studies. Each diagram shows the location of the SMA, CCZ, RCZ, pgACC, and sgACC. Motor areas are filled or outlined in yellow; medial prefrontal areas are filled or outlined in blue. (*C*) Sympathetic-related activations, red circles (36 studies, see *SI Materials and Methods* for the studies included). (*D*) Negative affect, red circles (data from ref. 31). (*E*) Cognitive control, red circles (data from ref. 31). (*F*) Sites of deep brain stimulation (DBS) for treatment-resistant depression, white pluses (data from ref. 33); sites of activation associated with meditation, red circles (see *SI Materials and Methods* for studies included). CA-CP, red horizontal lines; Vca; red vertical lines.

output originate from the same cortical areas that are involved in the preparation and generation of skeletomotor output. The colocalization of visceromotor and skeletomotor function may provide a mechanism to facilitate the coordination of these two motor systems (Fig. 5).

Second, we found that the top-down influence over the adrenal medulla by M1 originates largely from its trunk representation. This region of M1 projects to thoracic segments of the spinal cord (27). These spinal segments also contain the sympathetic preganglionic neurons that form the final common pathway to the adrenal medulla (10). These observations suggest that there is a link between the descending control of "core muscles" and the regulation of sympathetic output. This link could provide a neural substrate for the control of stress through "core" exercises, such as yoga and pilates (28).

Third, our results indicate that a major component of the cortical influence over the adrenal medulla arises from multiple motor and nonmotor areas on the medial wall of the hemisphere (Figs. 2, 3, and 5*B*). This finding differs from the view that autonomic outflow from the cingulate cortex originates from a single region, the subgenual ACC (22). Our perspective is supported by functional imaging data from human studies that show clusters of activations associated with sympathetic arousal in multiple areas of the cingulate cortex including the RCZ, CCZ, and pregenual ACC (Fig. 4*C*).

The human counterparts of the CMAr, CMAv, CMAd, and pregenual and subgenual ACC (Fig. 4 *A* and *B*) all display activation in tasks that are associated with negative affect (Fig. 4*D*). The RCZ of humans and the CMAr of monkeys are consistently activated in tasks requiring "cognitive control" such as response selection, conflict, and error awareness (Fig. 4*E*) (22, 29–32). In essence, our results provide the neural circuitry that links negative affect (e.g., sadness) and cognitive control processes (e.g., awareness of errors) to stress responses. The same substrate may mediate comparable stress responses when a sad situation or an error is recalled. Furthermore, abnormal activation of this circuitry may be fundamental to conditions like posttraumatic stress disorder.

The cortical areas we have identified as regulating the adrenal medulla overlap substantially with those included in "depression networks" (20, 21). One node in this network, subgenual ACC, displays histological changes in subjects with bipolar familial depression (21), and deep brain stimulation near subgenual ACC mitigates some symptoms of depression (20, 33) (Fig. 4*F*). Another node in the network, the pregenual ACC, is a site of activation during mindful meditation (Fig. 4*F*), a behavioral technique for reducing anxiety. Similarly, the pregenual ACC in monkeys appears to be uniquely linked with reward-related systems in the basal ganglia (34) and to be involved in regulating anxiety and emotional valence while deciding on a course of action (22, 35–37). Taken together, these findings raise the possibility that the areas of the cerebral cortex that influence the adrenal medulla (Fig. 5) also are key cortical nodes of a "stress and depression connectome." Perhaps some of these nodes represent additional targets for therapeutic intervention in affective disorders.

In summary, the control of the adrenal medulla is embedded in cortical areas that are involved in the control of movement, cognition and affect (Fig. 5). This means that mental operations like the preparation to move, the appreciation of conflict and feelings of sadness may be linked to the regulation of adrenal function. The cerebral cortex is a site of neural plasticity and information storage. As a consequence, aberrant plasticity could be a cause of or contribute to psychosomatic illnesses, just as the engagement of cortical plasticity through moving, thinking and feeling may help to cure or ameliorate them.

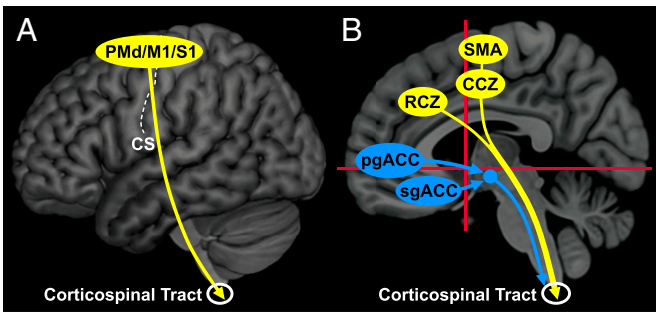

Fig. 5. Origin and pathways for top-down influence over the adrenal medulla by the cerebral cortex. Motor areas are filled yellow, and medial prefrontal areas are filled blue. (*A*) Major cortical areas for top-down control on the lateral surface of the hemisphere. All of these areas send projections to the spinal cord. (*B*) Major cortical areas for top-down control on the medial wall of the hemisphere. The motor areas send projections to the spinal cord, whereas the output of medial prefrontal areas is likely to be mediated by various subcortical centers. CA-CP, red horizontal lines; Vca; red vertical lines.

Materials and Methods

This report is based on observations from Cebus monkeys (1.4–3.7 kg; 3 male, 6 female), which received injections of RV (CVS-N2c; 5.0×10^8 pfu/mL) into the adrenal medulla. RV was especially useful for these experiments because current evidence indicates that RV is transported transneuronally in all types of systems and across all types of synapses (38–40). In addition, there is no evidence that the N2c strain is transported more efficiently in some pathways than others.

All procedures were in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by both the Institutional Animal Care and Use Committee and the Biosafety Committee at the University of Pittsburgh. Biosafety practices conformed to Biosafety Level 2 regulations outlined in *Biosafety in Microbiological and Biomedical Laboratories* (41). Procedural details for handling virus and virus-infected animals have been published (38).

Adrenal Medulla Injections. Surgeries were performed under general anesthesia [15 mg/kg Ketamine IM for sedation and 1–3% (vol/vol) isoflurane in 1–3 liters/min O_2 for general anesthesia] and aseptic conditions. Analgesics (buprenorphine 0.01–0.03 mg/kg s.c.) were given perioperatively. The left adrenal medulla was accessed via a paralumbar incision. We placed multiple RV injections (total of 80–100 μL) throughout the central regions of the adrenal gland by using a Hamilton microsyringe with a 30-gauge needle. The injection syringe was held in place for 30 s. After removal of the injection needle, the site was blotted with a sterile cotton swab to prevent any leakage through the injection tract. Following the injections, the wound was sutured, and the animal was returned to a cage designed for housing virus-infected animals.

Survival Period. We varied the survival times from 97 to 136 h. The mean survival times were 120.2 h (fourth-order labeling, $n = 4$), 126.8 h (fifth-order labeling, $n = 2$), and 131.5 h (sixth-order labeling, $n = 2$). At the end of the survival period, each animal was sedated with ketamine (25 mg/kg), deeply anesthetized with sodium pentobarbital (40 mg/kg IP), and perfused transcardially with three solutions: (*i*) 0.1 M phosphate buffer, (*ii*) formalin (3.7 gm formaldehyde gas/100 ml of 0.1 M phosphate buffer), and (*iii*) 10% (vol/vol) glycerol. Two to three liters of each solution was delivered over a period of 7–10 min per solution. After perfusion, the brains and spinal cords were removed and stored at 4 °C in 10% (vol/vol) phosphate-buffered formalin with 20% (vol/vol) glycerol.

Histological Procedures. We cut serial frozen sections (50 μm) in the coronal plane of a brain block including the cerebral cortex from its rostral pole to the parietal-occipital sulcus. The cerebellum and brainstem were cut separately. We also cut serial frozen sections (50 μm) of a spinal cord block containing the fourth thoracic segment (T4) to the first lumbar segment (L1) in the transverse plane. We stained every 10th section of the brain and every 20th of the spinal cord with cresyl violet to enable analysis of cytoarchitecture. To identify virus-infected neurons, we performed immunohistochemical reactions on free-floating sections according to the avidin-biotin peroxidase method (Vectastain; Vector Laboratories). The reactions used a mouse monoclonal antibody (M957, diluted 1:300; supplied by A. Wandeler, Animal Disease Research Institute, Nepean, ON, Canada) that is specific for the P antigen expressed by RV (42). All reacted sections were mounted on gelatin-coated glass slides, air-dried, and coverslipped with Cytoseal.

Analytic Procedures. Sections through the entire brain (every fourth or eighth) and spinal cord (every 10th) were examined under the microscope for reaction product by using brightfield and/or polarized light illumination. Another series of sections (every 10th) was stained and examined for cytoarchitecture. We plotted section outlines, labeled neurons, gray-white matter boundaries, cytoarchitectonic borders, and other anatomic features by using a computer-based charting system (MD2 or MD3; Accustage). Then, we used these plots along with software written in the laboratory to create unfolded cortical maps that displayed the distribution of labeled neurons, cortical sulci, and cytoarchitectonic borders on a 2D surface. The procedures used to unfold the cortex and generate maps of the lateral surface and medial wall of the hemisphere have been described (11). The criteria we used to define cytoarchitectonic borders in the cebus monkey have been presented in prior studies (refs. 12 and 43; see also ref. 44). To create the composite maps (Fig. 2 *C* and *D*), we overlaid the unfolded maps from four of the fourth-order animals. Then, the unfolded maps of the lateral surface were aligned on the central and arcuate sulci and the medial edge of the hemisphere. The maps of the medial wall were aligned on the anterior posterior extent of the corpus callosum and the medial edge of the hemisphere. The maps of individual animals required only small shifts (<1 mm) in the anterior-posterior and medio-lateral directions to achieve a best fit.

Determining Order of Transport. Survival time, in and of itself, is not sufficient to define the number of synapses mediating transneuronal transport (i.e., order of transport) in any experiment. Instead, we use the distribution of infected neurons within well-established pathways as internal controls (Fig. 1, *Right*). Specifically, after injections of rabies into the adrenal medulla, we determined the order of transport based on the following patterns of labeling: third-order transport, infected neurons are first seen in layer V of the cerebral cortex; fourth-order transport, infected neurons are first seen in cortical layers outside of layer V and in the deep cerebellar nuclei; fifth-order transport, infected neurons are first seen in cerebellar cortex (i.e., Purkinje cells); and sixth-order transport, infected neurons are first seen in the inferior olive.

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