Neural Networks of the Mouse Neocortex

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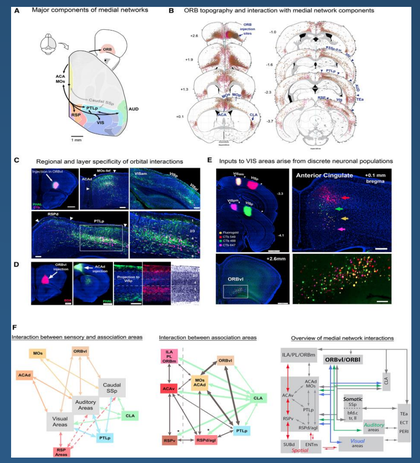
Introduction

Numerous studies have examined the neuronal inputs and outputs of many areas within the mammalian cerebral cortex, but how these areas are organized into neural networks that communicate across the entire cortex is unclear. Over 600 labeled neuronal pathways acquired from tracer injections placed across the entire mouse neocortex enabled us to generate a cortical connectivity atlas. A total of 240 intracortical connections were manually reconstructed within a common neuroanatomic framework, forming a cortico-cortical connectivity map that facilitates comparison of connections from different cortical targets. Connectivity matrices were generated to provide an overview of all intracortical connections and subnetwork clusterings. The connectivity matrices and cortical map revealed that the entire cortex is organized into four somatic sensorimotor, two medial, and two lateral subnetworks that display unique topologies and can interact through select cortical areas. Together, the data provides a resource that can be used to further investigate cortical networks and their corresponding functions.



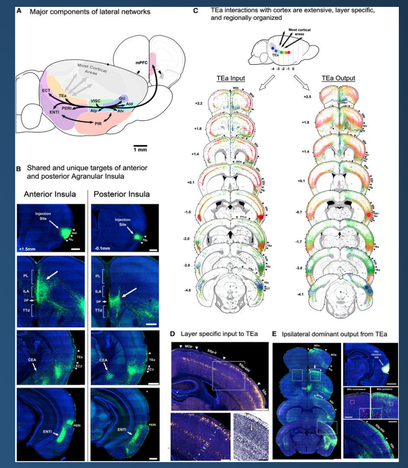


Figure 5. The Lateral Subnetworks:
(A) Sagittal view of the major components of two lateral subnetworks: the anterolateral insular (including the AId, AIv, AIp, VISC, GU) and posterior temporal (including TEa, ECT, PERI). (B) Distinct projection patterns of the anterior agranular areas (PHAL injections involved both AId and AIv, left panel) and AIp (right panel) with the mPFC areas (PL, ILA, DP), posterior temporal areas (TEa, ECT, PERI), and ENTl. The AIp targets more ventral structures in mPFC and more heavily innervates the central nucleus of the amygdala (CEA). Scale bars, 500 mm. (C) Map of neuronal inputs to (left) and output from (right) the TEa, which are arranged topographically along the rostrocaudal direction. Retrogradely labeled neurons are indicated as colored dots and demonstrate the layer-specific origin of cortical projections to TEa. Axonal pathways arising from TEa (outputs) are rendered as shaded areas of color. (D) Raw image of retrograde labeling (FG, yellow) following injection in TEa. Cells are distributed extensively across numerous cortical regions following a single, small injection, suggesting a high level of convergence. Bottom left panel shows close up of layer specificity in somatosensory barrel field, with most cell bodies residing in layers 2/3, 5a, and some layer 6. Fluorescent Nissl inverted (right) to aid in discriminating layers. Layer 4 "barrels" indicated with arrow. Scale bars, 500 mm (top) and 200 mm (bottom). (E) Raw image of coinjection in TEa. Fibers are predominately ipsilateral, but retrogradely labeled inputs are evenly distributed across both hemispheres.

Results

Data Production and Collection

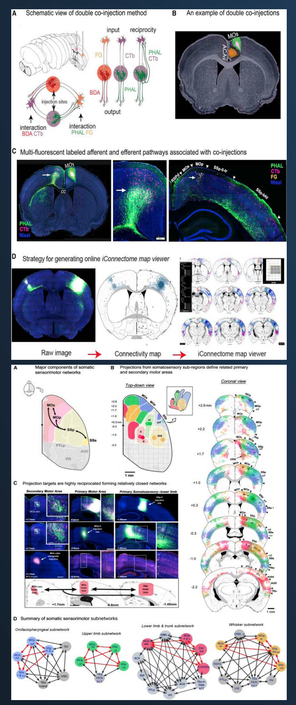


Figure 1. Strategy for Generating the Cortical Connectivity Atlas:
(A) Schematic illustrating a PHAL/CTb and BDA/FG double coinjection in two different structures labeling both input to, and output from each injection site. Reciprocal interactions between brain regions and circuit interactions between each injection site may also be revealed. (B) A coronal section showing coinjections made into the MOs and ACAv viewed with Nissl background to reveal cytoarchitecture. Scale bar, 1 mm. (C) Intermixed anterogradely labeled axons (green, PHAL) and retrogradely labeled neurons (pink, CTb) in the MOs following a coinjection in the contralateral hemisphere (first two panels, arrows). Note: the PHAL/CTb coinjection is the same as pictured in B. Image histogram was adjusted differently for the two hemispheres so that PHAL/CTb labeling on the left side can be viewed properly without over exposing the injection site on the right side. Last panel, comparison of retrogradely labeled neurons from injection in ACAv (arrow, yellow, Fluorogold [FG]) and fibers and cells from injection in MOs (PHAL/CTb). Fluorescent Nissl in blue; scale bar, 200 mm. (D) Strategy for mapping fluorescent labeling from a raw image (left, scale bar, 1 mm) onto the corresponding level of the ARA (middle) to generate a comprehensive map of projection pathways for all injection sites (right). Note: anterogradely labeled pathways were rendered as layer and regional-specific shading, while retrogradely labeled neurons were represented by individual dots. The large circle on the right hemisphere represents an injection site.

Figure 3. The Somatic Sensorimotor Subnetworks:
(A) Overview of the four major components of somatic sensorimotor areas (SSp, SSs, MOp, MOs). Each region is extensively interconnected with all others. (B) Projections from representative injection sites (colored dots) in each of four basic body representations in primary somatosensory cortex: orofaciopharyngeal (orf, blue), upper limb (ul, green), lower limb and trunk (ll/tr, red), and whisker-related caudomedial barrel field (bfd.cm, yellow). Projection data in top-down view (left) were drawn to scale using coronal sections (right) and shaded regions represent the areal extent of the most dense projections from each of the injected regions. (C) Projections from each of the somatosensory subregions define presumably functionally related MOs and MOp subregions, which are tightly reciprocated, as indicated by closely overlapped axonal fibers and retrogradely labeled cell bodies following coinjection. (D) Building on these observations, four network graphs were created using each of the defined somatosensory regions as starting points. Each subnetwork is distinct and all components within it share a high degree of interconnection. Each are composed of several somatic sensorimotor "nodes" (color coded to match anatomically defined functional domains in (B) that are reciprocally connected (as indicated with red arrows). Each of these subnetworks also includes other nonsomatic "peripheral" nodes (gray circles) and their connections are shown with gray arrows.

Connectivity Matrices

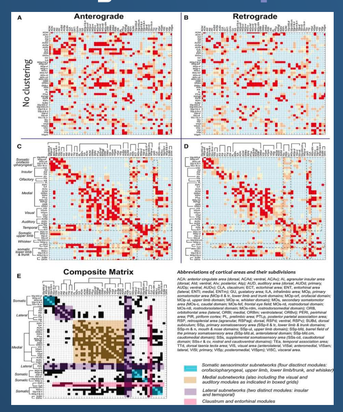


Figure 2. Weighted and Directed Cortico-Cortical Connectivity Matrices: (A–E) Connectivity matrices were constructed based on either anterograde (PHAL, A) or retrograde (FG/CTb, B) tract tracing data. In both matrices, connection origin is listed along the row while targets are listed across the columns (sorted alphabetically). The weighting of each connection is indicated by red (strong), orange (moderate), and yellow (light) coloring. In (C) and (D), the anatomical data in (A) and (B) has been reordered, illustrating a total of 12 distinct modules in different cortical subnetworks. Combining retrograde and anterograde tracing methods formed the composite matrix (E), a consensus perspective of corticocortical subnetwork connectivity.

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Figure 4. The Medial Subnetworks:
(A) Major components of the medial subnetworks, which mediate transduction of information between sensory areas (VIS, AUD, and caudal-most SSp) and higher-order association areas along the medial bank of the neocortex, such as the retrosplenial (RSP), parietal (PTLp), anterior cingulate (ACA), and orbital (ORB) areas. (B) Connectivity pathways of the medial subnetwork revealed by coinjections in the ORB. (C) Representative raw images from an ORBvl coinjection. (D) Laminar-specific differences in axonal projections to primary visual cortex (VISp) arising from either ORBvl (red, BDA labeling) or ACAd (green, PHAL). (E) Four different retrograde tracers were injected into the VISp (two), VISam, and VISpm within the same brain, resulting in distinct, topographically arranged clusters of neurons in the ACA and adjacent MOs-fef. In the ORBvl, these retrogradely labeled neurons are intermixed, but mostly not colocalized. (F) Summary of interactions among the medial subnetworks. Middle, connections between the association areas. Thicker arrows indicate dense projection patterns between regions. Dashed line separates a direct pathway to medial prefrontal region along the ventro-medial bank of the cortex (second medial subnetwork). Asterisks indicate a unidirectional connection between CLA and RSP. Right panel, overview of medial network interactions including TEa and parahippocampal structures (i.e., SUBd, ENTm), which project to RSP (red arrows). Reciprocal connections of visual (blue) and auditory (green) areas with all major medial network components shown. Caudal-most somatosensory areas (SSp-ll/tr; SSp-bfd.cm) are included as well (gray arrows).

Network for the Mouse Neocortex

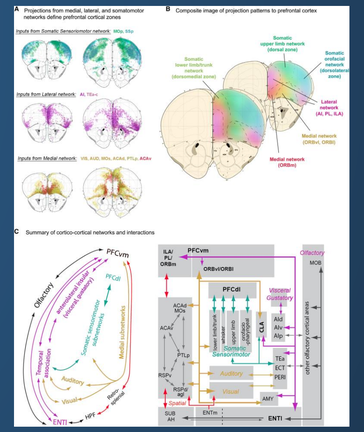

Figure 6. Interactions with Prefrontal Cortex:
(A and B) Cumulative projection from components of the somatic sensorimotor, lateral, and medial networks in two representative coronal sections of the prefrontal cortex (PFC). Collectively these represent inputs from the entire neocortex to the PFC. Inputs were color coded based on the location of the injection sites in different components of the network (A). For example all primary motor projections arising from multiple injections along the length of this structure were colored green and all somatosensory projections were colored blue (A, top). ACAv was colored red to separate it as a component of the second medial subnetwork (A, bottom). (B) All inputs from three somatic sensorimotor subnetworks (as shown in A) converge onto three distinct zones, dorsolateral (dl), dorsal (d), and dorsal medial (dm), in the dorsolateral half of the prefrontal cortex (PFCdl, green and blue). In contrast, the medial and lateral subnetworks converge onto the ventromedial half of the prefrontal cortex with distinctive patterns. (C) A schematic view of cortico-cortical network information flow as seen in a top-down view of the cortex (left, lateral edge on left, PFC at the top). Right, a more detailed overview of these interactions (lateral edge of cortex on the right, PFC at the top). Somatic sensorimotor boxes are meant to include both the sensory area and its corresponding primary motor area with which it is strongly interconnected. All functionally distinctive subnetworks are organized along the longitudinal axis of the cerebrum. Information processed in the medial and lateral subnetworks is integrated within the ventromedial half of the prefrontal cortex (PFCvm) and the ENTl. The claustrum (CLA) may also provide an additional means of direct interaction between each of the subnetworks.

Discussion

We have demonstrated the feasibility of producing and collecting large-scale connectivity data; however, interpretation of this wealth of anatomical data presents an ongoing challenge. This resource provides a reference for determining the complete set of inputs and outputs for a given cortical region and for implicating it in a broader network context. Any of these long-range interactions may be validated at the synaptic level using transsynaptic viral tracing and may be further investigated to determine cell-type-specific connections or be assessed functionally using available optogenetic techniques.

1. Zingg B, Hintiryan H, Gou L, Song MY, Bay M, Bienkowski MS, Foster NN, Yamashita S, Bowman I, Toga AW & Dong HW 2014 Neural networks of the mouse neocortex. Cell, 156(5):1096-111.

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