Towards an understanding of the intrinsic biophyisical diversity and connectivity of mammalian neurons

Troy W. Margrie

Division of Neurophysiology, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA, London, United Kingdom.

A neuron's anatomical and functional connectivity, together with its intrinsic biophysical profile, defines its function. Although different classes of neurons are known to express different types and constellations of membrane channels, very few studies have quantified such biophysical diversity across the population of one neuronal class. We have recently explored this issue in principal olfactory bulb mitral cells both *in vivo* and *in vitro* and find the amount of hyperpolarisation-evoked membrane potential sag is highly variable, ranging from zero to more that 30 mV [1}. We also find that the amount of sag expressed profoundly impacts the input-output function of an individual cell [1]. Most notably though is the observation that the sag amplitude recorded in a given neuron is a biophysical fingerprint for all mitral cells belonging to the same functional network. Using a transgenic "monoclonal nose" mouse whereby all sensory input to the bulb is genetically and globally altered to express the same single type of odorant receptor [2], we also see that differences in network-based expression of mitral cell sag is dependent on the diversity of sensory inputs arriving from the periphery.

In parallel, we have also recently developed tools that enable us to assess the intrinsic profile and sensory function of an individual neuron *in vivo*, then to trace the upstream connectivity profile of that cell [3]. To achieve this we use *in vivo* whole-cell recordings to first record and quantify the intrinsic and synaptic receptive field properties of a neuron. Simultaneously, we deliver DNA vectors via the patch pipette to drive expression of the protein machinery required for single-cell rabies virus-mediated retrograde labeling of upstream neurons. Approximately two days after recording, the modified rabies virus is extracellularly injected in close proximity to the target neuron to specifically infect to the recorded cell, then spread –in a retrograde manner–across synaptic connections to its presynaptic cells. Two weeks after recording and virus injection, brains are mounted under a two-photon microscope that includes an integrated and automated x-y-z stage and vibratome for whole-brain serial sectioning (Figure 1). This fully automated device —that facilitates high-throughput analysis of large-scale datasets—significantly improves workflows such that entire adult mouse brains may be optically sectioned within reasonable timeframes. Image tiles and brain sections are then seamlessly re-stitched such that it is now realistic to begin the map the connectivity of neurons within and across brain regions and animals. This will allow us to establish a three-dimensional functional connectivity atlas of single cells and information pathways in the brain.

References

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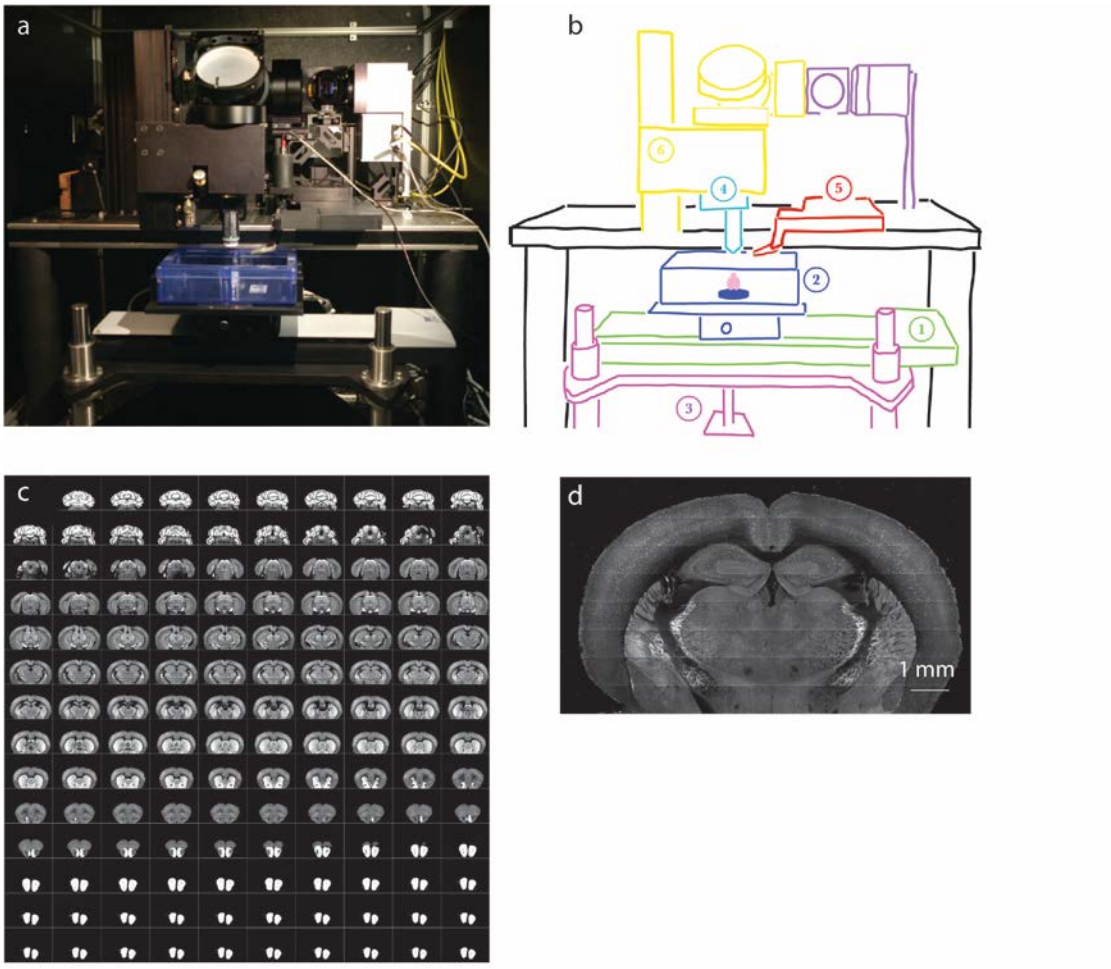

Figure 1: *Serial block-face two photon mocroscope for whole-brain sectioning. (**a**) Photo of microscope. (**b**) Cartoon of integrated components required for serial block-face sectioning: (1) linear (x) stage, (2) slicing chamber (y) stage, (3) z-stage, (4) objective piezo (z), (5) vibratome, (6) 2p microscope. (**c**)Example image series from a GAD-67 GFP mouse whereby each physical coronal section is 100 microns thick starting from anterior (olfactory bulb; bottom right) to posterior (cerebellum; top left). (**d**) A high magnification atlas view of a single image plane comprised of 11 x 16 tiles (832 x 832 pixels) (image cropped).*