Whole-brain density of neurons projecting to VTA dopaminergic neurons

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Abstract

Recent developments reported in (Watabe-Uchida2012) allowed to obtain a complete list of monosynaptic inputs to midbrain dopaminergic neurons. The technique involves injection of the modified rabies virus SAD DeltaG-GFP(EnvA) into the Ventral Tegmental Area (VTA) and Substantia Nigra_compact part (SNc) of transgenic mice that express Cre in dopamine neurons. The Cre/loxP recombination system controls the expression of the cognate receptor TVA and of the rabies-virus envelope glycogene protein (RG), thus allowing selective initial infection of dopaminergic neurons, and transsynaptic transfer from them.

So far, projections to dopaminergic neurons have been worked out by manually counting fluorescently-labelled neurons section by section. In particular [Watabe-Uchida2012] established the existence of direct projections from striatal neurons to VTA and SNc dopamine neurons.

On the other hand, the Mouse Brain Architecture project (MBA) aims to construct a whole-brain wiring diagram of mouse, at a mesoscopic scale corresponding to brain regions in classical neuroanatomy. In the MBA pipeline, injected brains are cryosectionned using a tape-transfer method, and imaged using a nanozoomer. Gigapixel images of brain sections have been co-registered to the Allen Reference Atlas and posted on the Web portal `www.brainarchitecture.org`. Moreover, a computational pipeline allows to reconstruct processes of neurons in three dimensions.

Mouse preparation

We injected five mouse with modified AAV brains and processed them through the MBA pipeline. This results in an automated estimate of the three-dimensional coordinates of neurons projecting to dopaminergic neurons in VTA and SNc. As the brain sections are co-registered to the Allen Reference Atlas, the corresponding brain regions can be read off from the coordinates. This approach combines the neuron-type-specificity of the connectivity study [Watabe-Uchida2012], and the automated brain-wide coverage of the MBA project.

Sectioning and imaging MBA pipeline

- Brains are sectioned coronally and tape-transferred.
- Sections are alternatively Nissl-stained and fluorescently imaged using a Nanozoomer.
- After quality control, translations, rotations and dilations are applied to the stack of coronal sections to register them together (see Figure 4 for maximal-intensity projection of a registered brain, after filetring out artefacts outside tissue).

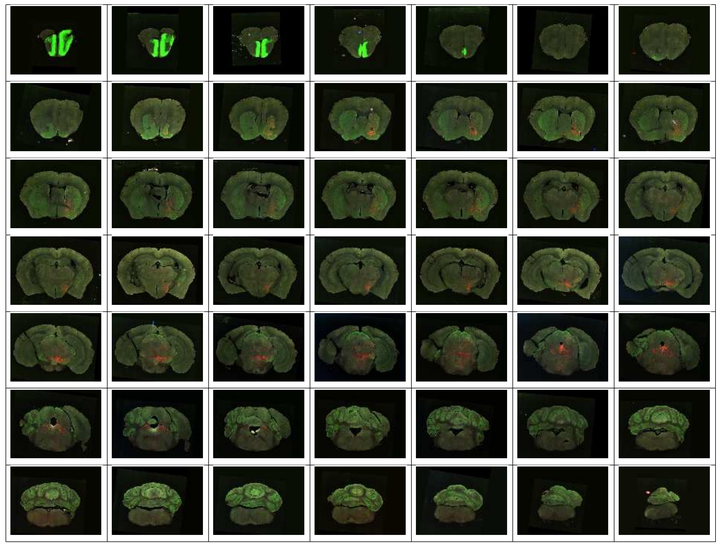

Figure 2: **A stack of fluorescently imaged coronal sections, regularly spaced by 240 microns (this is a subset of 260 sections from the brain show in Figure 3.**

Reconstruction of brain from raw data

The imaged, coronally sectioned brains, clearly show colored signal, which at finer resolutions allow for cell segmentation and counting (a total of 1440 red-labeled cells are detected from the fluorescently imaged sections, which leads to $\simeq$ 3,000 cells in the brain to account for Nissl sections).

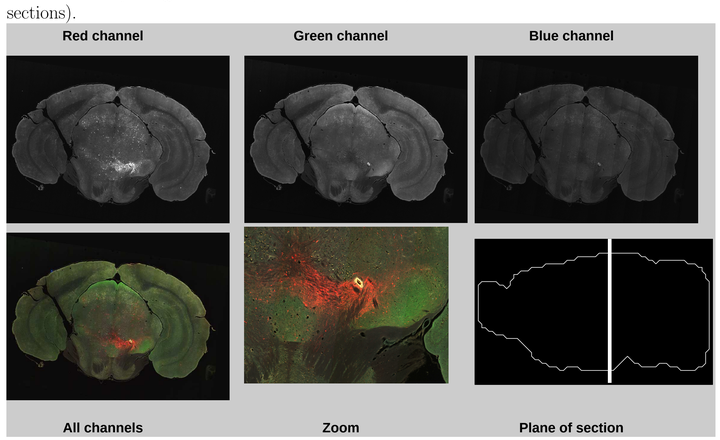

Figure 3: **Separate channels in the coronal section corresponding to the maximum intensity of the red channel inside the tissue across a whole brain. The plane of section through the ARA is shown on the right-hand-side.**

Stacks of inages are registered together by applying rotations and translations to coronal sections in order to optimize the firt to the three-dimensional profile of the mouse brain in the Allen Reference Atlas.

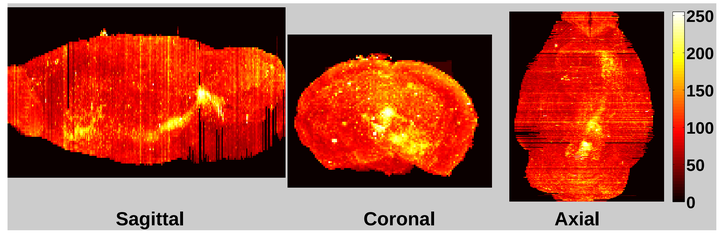

Figure 4: **Maximal-intensity projections of the reconstructed volume based on a registered stack of the red channel Jpeg images for one brain.**

Automated brain-wide cell detection, and comparison to neuroanatomy

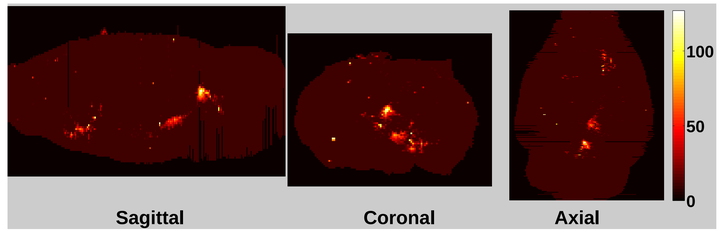

Figure 5: **Maximal-intensity projections of numbers of cells detected from in the red channel (voxel side = 100 microns). The tissue area is represented in deep red (value 10 in the 'hot' colormap of MATLAB 2011).**

Given an imaged brain, the registration pipeline pegs a list of landmarks of the brain (such as ventricles, cusps in the boundary of a coronal sections, cerebellar folds), to the atlas space, which induces a deformation field from the brain images to the atlas space. From the coordinates of the detected cells in real space (given by the center of mass of the pixels belonging to the cell in a coronal section, together with the position of the coronal section), the registration allows one to read off in which brain region; 126 regions have at least one cell in them. The top regions ordered by number of cells are illustrated in Figure 7.

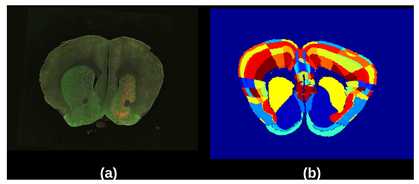

Figure 6: **(a) The 59th fluorescently imaged section (out of 260). (b) The result of the pixel-by-pixel mapping to atlas space. The uniform colors correspond to numerical ids of brain regions in the hierarchical ARA (2011 version, consisting of 1198 regions).**

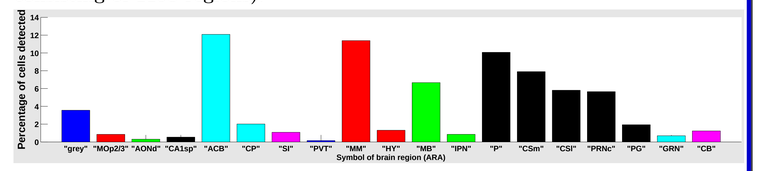

Figure 7: **Contributions of 13 main brain regions in the ARA to cell count (best subregions or subregions contributing more than 2 %). The largest contributions come from striatum, hypothalamus, midbrain and pons.** The acronyms read 'grey' = 'Basic cell groups and regions', MOp2/3 = Primary motor area Layer 2/3, ACB = Nucleus Accumbens, AONd = Anterior olfactory nucleus dorsal part, CA1sp = Field CA1 pyramidal layer, CP = Caudoputamen, SI = Substantia innominata, PVT = Paraventricular nucleus of the thalamus, MM = Medial mammillary nucleus, HY = Hypothalamus, MB = Midbrain, IPN = Interpeduncular nucleus, P = Pons, CSm = Superior central nucleus raphé medial part, CSl = Superior central nucleus raphé lateral part, PRNc = Pontine reticular nucleus (caudal part), PG = Pontine gray, GRN = Gigantocellular reticular nucleus, CB = cerebellum.

Density of midbrain dopaminergic neurons from the Allen Atlas of the adult mouse brain

The transcriptomes of A9 and A10 dopaminergic neurons have been obtained in (Chung2005). Given a set of $T = 64$ cell-type-specific transcriptomes, we estimated the brain-wide density profile of dopaminergic neurons (Grange2013):

$$\text{Allen Atlas}(\text{voxel}, \text{gene}) = \sum_{\text{type}} f(\text{voxel}, \text{type}) C(\text{type}, \text{gene}) \qquad (1)$$

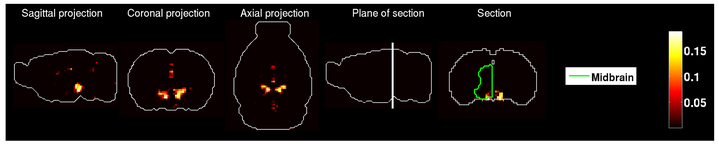

Figure 8: **Estimated brain-wide density of A9 dopaminergic neurons.**

Acknowledgments

We gratefully acknowledge funding support from the NIH (Directors Transformative Award MH087988, DA036400), NSF (Inspire Award PHY-1344069), the Mathers Foundation, the Crick-Clay Professorship to Dr Mitra and CSHL internal funding.

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(ARA) Website: 2012 Allen Institute for Brain Science. Allen Mouse Brain Atlas [Internet]. Available from: `http://mouse.brain-map.org/`