CONNECTOMICS: A JOURNEY THROUGH TIME.

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ABSTRACT

Connectomics is a relatively new field in neuroscience which tackles the intricate neural circuitry of the brain (1). Several technologies have been constructed along the years to aid in visualising the central nervous system and in taking on the challenge of obtaining a structural map of the brain's neural connections (2). This review goes over the major milestones reached in this field from the first full connectome ever obtained, the Brainbow technique, and eventually, the collaborative approach launched by the National Institutes of Health, i.e. the Human Connectome Project. This ambitious project aims to establish how brain connectivity underlies brain function by compiling brain imaging data and freely sharing this with researchers from all over the world (3). Enhancing our knowledge of brain structure and function, possibly through such collaborations, will hopefully allow insight into the processes underlying behaviour, and possibly, a better understanding of brain diseases and disorders in the future (4).

LIST OF ABBREVIATIONS

Alzheimer's disease (AD)
Caenorhabditis elegans (*C. elegans*)
Connectome Coordination Facility (CCF)
Human Connectome Project (HCP)
Lifespan Connectome Studies (LCS)
Magnetic Resonance Imaging (MRI)
Massachusetts General Hospital (MGH)
National Institutes of Health (NIH)
Resting state-functional magnetic resonance imaging (rfMRI)
Spectral and photophysical protein variants (XFPs)
Task-based functional magnetic resonance imaging (tfMRI)
University of California Los Angeles (UCLA)
Washington University, the University of Minnesota (UMinn) and Oxford
University consortium (Wu-Minn-Ox)

OVERVIEW OF THE FIELD OF CONNECTOMICS

This century has been renowned for its major leaps in science, particularly the accomplishment of the Human Genome Project in sequencing the entire human genome in 2001 (5,6). Analogously, a major challenge which neuroscientists are confronting involves mapping the connections of the brain with the hope of unravelling the secrets of the human mind (1). The desinence of the word 'connectomics' is the same as that of the word 'genomics', and as genomics involved the sequencing of the entire set of genes (the genome) of several species, connectomics aims to map all of neuronal connections of the brain (the connectome) to allow a better understanding of human brain function (7).

'Connectomics' was better defined by Lichtman and Sanes as "a branch of biotechnology concerned with applying the techniques of computer-assisted image acquisition and analysis to the structural mapping of sets of neural circuits or to the complete nervous system of selected organisms using high-speed methods, with organizing the results in databases, and with applications of the data" (8). Therefore, the analysis of connectomes requires new developments in technology (1). Novel biotechnologies were, in fact, developed for this endeavour aimed at generating circuit diagrams at macroscopic, mesoscopic or microscopic resolutions (2).

Continuous innovation in new technologies is expected to help pave the way for a more in-depth understanding of the brain's complex neural connections. Understandably, along with the study of the normal human connectome comes the study of its disturbances, so-called connectopathies, which are most definitely associated with numerous neurological (9) and psychiatric disorders (8). Hence, obtaining connectome data will facilitate the study of brain disorders, including schizophrenia, autism and epilepsy (10–12), from a network perspective (13) and possibly result in improved treatments from which society will benefit greatly (14). Furthermore, through collaborations, researchers can accelerate the progress towards understanding the processes that underlie brain function and brain circuit formation, and lead to the heightening of our knowledge of growth, plasticity (15), consciousness and intelligence (14).

THE FIRST FULL CONNECTOME - CAENORHABDITIS ELEGANS

The ground-breaking paper, "The structure of the nervous system of the nematode Caenorhabditis elegans", also known as "the mind of a worm" (16), was the first ever documentation of the entire nervous system of an animal. This paper signified the end of the first phase of a project initiated around twenty years prior by Sydney Brenner. This project ambitiously began to confront the enigma that is the brain, and through this, Brenner and his colleagues founded the field of connectomics, a field which was yet to be named at their time (17).

Brenner believed that by knowing the structure of the nervous system and the pattern of its connections, two further problems would remain: the genetic control of the nervous system and the control of the nervous system over behaviour (18). Although a structural description of the neural circuitry would not be fully sufficient as an answer to these problems, it was essential (19).

In 1963, Brenner acquired a culture of the nematode *Caenorhabditis elegans* (*C. elegans*) and left it in the hands of Nichol Thomson for him to section, fix and examine under the light microscope. To their satisfaction, a clear visual of the nervous system appeared, and *C. elegans* was chosen for this project. The identical cellular makeup of every individual organism, along with only 300 neurons making up its nervous system, its transparent body, good genetics, and short life cycle of around three and a half days made *C. elegans* ideal for this mission (1,17).

The next step involved obtaining a detailed synaptic map and the only way this could be done was through electron microscopy (14). Despite Brenner's attempts at obtaining machines and devising contraptions to facilitate the process, eventually, the reconstruction had to be done by hand (16). This involved marking glossy prints of electron micrographs from the series of sections obtained by Thomson, thus generating trails of coloured numbers, each indicating a different neuron. Finally, synapses were recorded and positioned on neuron maps which were presented (17).

This approach did not produce a full-scale three-dimensional representation, but rather skeleton maps which were sufficient to produce a proper circuit diagram of the nematode. The project took 15 years to complete (17).

A TECHNICOLOUR ADVANCEMENT – BRAINBOW

Cajal's revolutionary method of labelling neurons to detect cellular elements making up the neural circuit had one major limitation: it was only applicable to a small quantity of cells. The thought behind it, however, remained intriguing (20).

In 2007, a team led by Jeff Lichtman and Joshua Sanes at Harvard University, designed two genetic strategies, eloquently named 'Brainbow', for the stochastic expression of multiple spectral and photophysical protein variants (XFPs) on a single transgene. The expression of three different XFPs in combination would theoretically result in the coloration of neurons in one of ten hues. Yet if these three XFPs are expressed to different degrees in different neurons, a much greater number of hues will result.

The stochastic expression of colour was accomplished through a mechanism based on the Cre/loxP recombination scheme. Cre recombinase selectively catalyses recombination between a pair of so-called loxP sites, each consisting of 34-nucleotide sequences. Subsequently, a DNA segment that lies in between two loxP sites of the same orientation is excised, whilst a DNA segment lying in between two loxP sites of opposite orientation is inverted (21). Hence, this technique allows for the discrimination of individual neurons on the basis of colour in over 100 colours rather than just two or three (20).

The ability to delineate individual neurons and trace their processes within their native context in the central and peripheral nervous systems of transgenic mice, expedites the study of how these neurons interact. Therefore, it has allowed researchers to make a huge leap towards their ultimate goal which aims to comprehend how neural circuits underlie behaviour (22).

Just like any other technique, the Brainbow method has its limitations. Primarily, a spectrum of 100 colours is still insufficient to view full regions of the nervous system (1,21). Moreover, due to the diffraction-limited resolution of light microscopy, fluorescence images appear at a relatively low resolution when observing more complex neuronal wiring systems. Hence, thinner sections are required. Fortunately, however, various solutions have already emerged, such as the use of Array Tomography which allows quantitative, large-field volumetric imaging of large amounts of XFPs and tissue molecular structure at high spatial resolution (23).

Despite its limitations, Brainbow contributed extensively to the field of connectomics since obtaining partial connectomes can also be of great insight into the complexity of the brain. In addition, partial connectomes can also be of great use in the study of mouse models of psychiatric disorders as it is being suspected that defects in neuronal connections, whether they concern pattern, number, or proportion, might underlie behavioural disorders, such as schizophrenia and autism. These disorders might arise due to genetic and environmental factors that can affect several developmental events, resulting in a quantitative or qualitative defect in circuitry, resulting in so-called 'connectopathies'. The review of quantitative and qualitative properties of brain circuitry through Brainbow labelling might be adequate to put this hypothesis up to the test (21).

THE HUMAN CONNECTOME PROJECT

Recent advances in neuroimaging have made the thorough study of in-vivo human brain connectivity possible across large numbers of individuals (24). By systematically compiling brain imaging data from numerous subjects and freely sharing this valuable information with others around the world, researchers can obtain insight into how brain connectivity underlies brain function in a shorter period (3). This will hopefully lead to improved diagnoses and treatments of brain disorders in the future (4). With these principal aims in mind, in 2009, the NIH Blueprint for Neuroscience Research inaugurated a $30 million project: the Human Connectome Project (HCP), with the intention of mapping the brain circuitry using revolutionary non-invasive brain imaging technologies (3).

In 2010, Blueprint awarded two research consortia with a sum of $40 million to collaboratively compile a wiring diagram of the human brain in high resolution (25). The Wu-Minn-Ox consortium aimed to comprehensively map the connectivity diagrams in each of 1200 healthy adults – twin pairs and their siblings, with a genetically-informative design, using ground-breaking techniques of non-invasive neuroimaging. This includes four magnetic resonance-based modalities, including a new 3 Tesla MRI scanner, as well as a new 7 Tesla scanner, along with magnetoencephalography and electroencephalogram (24).

The data acquired concerning the anatomical and functional connections between different regions of the brain of each individual would then be related to data obtained from behavioural testing. Through the comparison of connectomes and genetic information between identical twins and fraternal twins, the relative involvement of genes and environmental factors in the formation of brain circuitry could be determined. Moreover, further clarification of the organisation of brain networks could be obtained. After elaborate analysis using sophisticated tools, the data could then become accessible on the web through a customised Connectome Database Neuroinformatics Platform (4).

In collaboration, the MGH/Harvard-UCLA consortium focused on optimising MRI technology. This meant enhancing the resolution of diffusion MRI to unprecedented heights for even more accurate imaging of the structural connections of the brain (26).

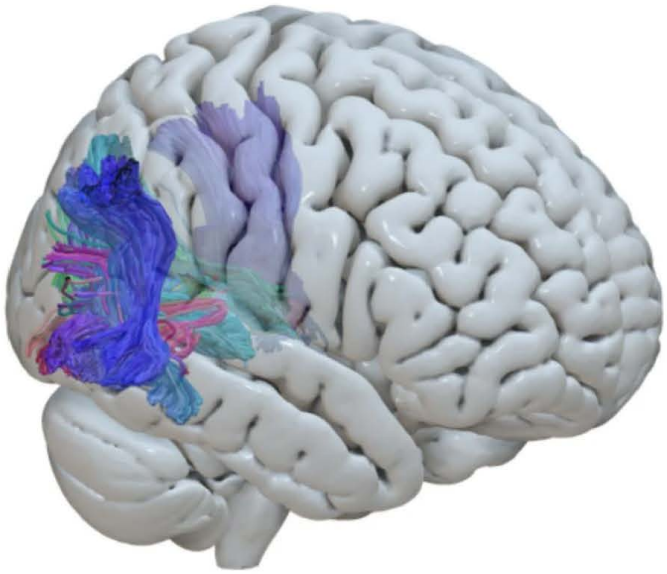

Figure 1: Connectivity in the occipital lobe generated from an average connectome from 24 individuals using Human Connectome Project data. Adapted from (21) CC-BY http://creativecommons.org/licenses/by/4.0/

Other initiatives around the world have been making even more connectome data available over the years, such as the Human Brain Project in Europe which aims to achieve the reconstruction of the multi-scale organisation of the brain, using simulation-based approaches along with productive loops of experiments and big-data analysis (27).

THE CONNECTOME COORDINATION FACILITY

Studies contributing to the HCP are housed within the Connectome Coordination Facility (CCF) (28). All CCF studies are classified under three main categories: Healthy Adult Connectome, Lifespan Connectome Studies and Connectomes in relation to Disease (29).

The study mentioned previously, titled HCP Young Adult, took on the challenge of charting the neuronal pathways from 1200 healthy young adults, using novel neuroimaging technology (30). Lifespan Connectome Studies (LCS) aim to extend the data gathered from HCP Young Adult to healthy humans of other age groups, to create a high-quality dataset to be used for comparison. Currently, there are four research projects concerned with LCS (31). Two of these large-scale brain imaging studies include the Human Connectome Projects in Development and Aging. These projects are collecting structural, rfMRI, tfMRI, diffusion, and perfusion MRI in participants from five to 100+ years of age. The result of this project will provide a rich, novel, and multi-modal dataset consistently collected across a wide age range (32). Earlier developmental stages are being researched by the Developing Human Connectome Project, which is researching prenatal and neonatal brain development, and the Lifespan Baby Connectome project which is studying brain development in children from birth to the age of five (28).

The third category of studies in the HCP: connectomes in relation to human disease, was funded by the NIH for HCP-style data collection to be applied to cohorts at risk for or already suffering from brain diseases or disorders. Over 10 disease connectome studies have been funded, and more are anticipated (28). These studies address many brain diseases and disorders, including schizophrenia and Alzheimer's disease (AD). Both schizophrenia and AD have been shown to have associated changes in brain connectivity throughout the course of their progression. Therefore, obtaining a large, accessible connectivity dataset from both healthy individuals and patients would allow further understanding of the correlation between these changes in connectivity and disease symptoms. In turn, this will benefit the prediction of prognosis and the treatment of the disease (33).

CONCLUSION

Despite the major advancements in the field of connectomics, some still question whether the efforts being put into the reconstruction of a connectome on the scale of a mammalian brain will be worth the large financial investment required, since one cannot be sure that the information obtained will be of any value. Many also argue that with the great amount of variability in the circuitry of the brain, it might not even be possible to relate structure to function. And if possible, it could be that our minds are no match for such complexity.

It is important to point out that connectomics does not dismiss the value of the information gained through physiological and pharmacological studies. It merely highlights the fact that the study of brain function without knowledge of its structure will be just as successful as the study of genetics without knowledge of the genome. Whilst connectomics data may not be the answer to every problem related to the understanding of the human mind, it may provide insight which might not be discovered otherwise. Through the comparison of healthy and diseased brains, young and old, and human and other primate brains, further knowledge could be gained of the foundations of psychiatric diseases, the developmental changes in circuitry, and a better understanding of intelligence.

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