THE IMPORTANCE OF STUDYING HUMAN BRAIN

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ZNAČAJ ISTRAŽIVANJA LJUDSKOG MOZGA

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ABSTRACT

Due to its numerous distinctive functions and unique pathology, the human brain, or rather forebrain has been difficult to study in common animal models. Although many basic molecular and cellular events are conserved across species, human brain connectivity, pertinent especially to the cerebral cortex circuitry, is unique and demands extensive research. Despite a great advancement in functional imaging methods accomplished over the last two decades, many basic features of healthy and diseased human forebrain remain elusive. Here we address difficulties in anatomical studies of developing and adult human brain and indicate the new directions and challenges to be addressed in the future. We pay special attention to possibilities of translating animal brain research to human cases. We consider that, although animal experiments play a vital role in understanding fundamental molecular and cellular mechanisms behind brain function, understanding of higher brain functions (language, intelligence, memory) has to be based on understanding uniqueness of human circuitries. Furthermore, brain is the site of many human-specific diseases, such as multiple sclerosis, schizophrenia, and Alzheimer's disease, for which only partial animal models exist. To study human brain, thus, remains irreplaceable in the quest for new therapeutic tools, as well as in understanding the essence of our being.

Keywords: *cerebral cortex, development, hippocampus, human, patient HM.*

SAŽETAK

Ljudski prednji mozak usled svojih brojnih karakterističnih funkcija i jedinstvene patologije teško je proučavati na uobičajenim životinjskim modelima. Iako su mnogi osnovni molekularni i ćelijski procesi prisutni u svim vrstama, veze ljudskog mozga, posebno kortikalne, jedinstvene su i zahtevaju opsežna istraživanja. Uprkos velikom napredku u funkcionalnim metodama snimanja (fNMR) ostvarenom u poslednje dve decenije, mnoge osnovne karakteristike zdravog i obolelog ljudskog prednjeg mozga ostale su nepoznate. Ovde se bavimo poteskoćama u anatomskim studijama ljudskog mozga, kako intrauterino tako i kod odraslih osoba i ukazujemo na nove pravce i izazove sa kojima će se budući istraživači susretati. Posebnu pažnju smo posvetili mogućnosti ekstrapolacije rezultata istraživanja sa životinjskih modela na ljuski mozak. Smatramo da, iako eksperimenti na životinjama igraju vitalnu ulogu u razumevanju osnovnih molekularnih i ćelijskih mehanizama koji stoje iza funkcije mozga, razumevanje viših moždanih funkcija (jezika, inteligencije, pamćenja) mora biti zasnovano na razumevanju jedinstvenosti ljudskih moždanih sinaptičkih kola. Štaviše, mozak je mesto nastanka mnogih bolesti specifičnih za ljude, poput multiple skleroze, šizofrenije i Alchajmerove bolesti, za koje postoje samo delimični životinjski modeli. Dakle, proučavanje ljudskog mozga ostaje ostaje nezamenjivo u potrazi zaa novim terapijskim pristupima, kao i u razumevanju suštine našeg bića.

Ključne reči: *moždana kora, razvoj, hipokampus, људски мозак, pacijent HM.*

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INTRODUCTION

It is in the nature of consciousness to try to uncover the origin of itself. Over the millennia human thought has evolved in giving an answer to this fundamental question through mystic, religion, art, philosophy and, particularly during the last few centuries, science. Neuroscience provides a unique perspective by placing consciousness inside of the brain (1).

Human brain is a unique structure in the known universe able to study itself. As the source of material for morphological studies of the human brain is sparse, numerous animal models have been used. Most notably, rodent species, mouse (Mus musculus) and rat (Rattus rattus) have been widely used in many other areas of research, as well as in neurosciences. Whereas the advantages of this reductionist approach are clear, these animals have relatively short gestation, numerous offspring, small size, simple diet, just to name a few advantages, there is an important caution when they are used for neuroscience research. Although all other organs and tissues in rodents are miniature versions of their human counterparts, it is not the case with the brain.

Based on this variation, neuroscientists have come up with a measure called the encephalization quotient (EQ) which describes brains size as a ratio of the expected average brains size for a given body weight (2). For example, humans have an EQ of about 7.5. This means that our brains are 7.5 times bigger than what one would expect for animals of our body size. In contrast, a squirrel has an EQ of 1.1, meaning that their brains are quite average for animals of their size. While this is somewhat controversial, some have argued that EQ correlates with the intelligence of a species. However, this is hard to determine because there is no good measure of intelligence in animals. Nevertheless, having a larger EQ does roughly correspond to having a more "developed" brain. For example, carnivores tend to have larger EQs than plant eaters, which is consistent with the fact that hunting is a more complex task than rummaging (3).

The most vigorously studied, rodent brains are lyssencephalic, a state which is incompatible with life in humans (4). Human brain, thus, undergoes much longer development during which it increases in size, develops gyri, and, functionally, has far greater capacity for information processing, memory, thinking, and, as a pinnacle – develops consciousness. It is beyond our scope to discuss the matter of uniqueness of processes such as the perception of time, feelings, self-consciousness and all other attributes of the human soul to our species, as the evidence to the contrary is sparse we will simply accepted this as an axiom for all further considerations. The question of extraterrestrial and other-dimensional conscious life forms will also not be considered, due to lack of data.

Other animal models, particularly studies of comparative neuroanatomy of mammals have been performed ever since Cajal grounded neuroscience (5). Especially important studies comparing mammalian brain with a perspective of human brain as the result of evolution have been published by Javier DeFelipe (6,7). Especially important in this context is study of non-human primate brains, as our closest evolutionary relatives (8, 9, 10, 11). Many ethical limitation, however, make this work in many ways even more difficult than work on human tissue, whereas the advantage is better and more even quality of tissue samples which are in primates obtained without post-mortem delay, as is always the case in humans.

Using several examples from the literature, we will try to build the case that, although experiments on animals are essential for the advancement of neurosciences, particularly in understanding molecular mechanisms behind physiological and pathological brain processes, parallel research on the human brain tissue samples is important as well.

METHODOLOGICAL REMARKS

Sparsely available human brain tissue for research purposes makes the possibilities for statistical analysis limited. Another problem is with the antibodies used to stain human brain tissue. Some of the usual ways of examining the specificity of antibodies in laboratory animals can simply not be applied to humans, particularly the "gold standard" method of showing the lack of immunostaining in knockout mice (12). As some antibodies do not cross-react across species, as the ultimate control for the specificity of staining – labeling of a knockout mouse tissue cannot be performed, reliability of available antibodies is difficult to prove. Other ways of controlling antibody specificity, all doable on human tissue, include the replacement of the primary antibody with the normal serum of animal in which the antibody was raised, and the immunoblot of the brain region. Another possibility is to confirm the expression with the *in situ* hybridization for mRNA of the protein to be probed and either double-label with the antibody or compare adjacent sections for the mRNA and protein expression. These controls are particularly important when working with the new antibody to describe the distribution of a previously unknown protein in human brain tissue (13). In general, a very important criterion is that all immunostained cells and structures have the appearance expected for the given antibody staining pattern (14, 15).

Larger developmental window, where many processes that in commonly used experimental animals take place within hours, or days, in human brain may take several weeks or even months (examples given below). Thus, studying human brain development, together with brains of non-human primates (where the availability of the tissue is almost equally complicated as in humans), may not only give insight into primate-specific developmental events, but also the previously unrecognized events ("missing links") in brain development shared by all mammals. Good example for the latter is that soon after the discovery of dorsally generated interneurons in humans (16), several publications reported similar developmental events in rodents (17, 18).

DEVELOPING HUMAN BRAIN

According to the disputed recapitulation theory embryonic development recapitulates evolution (19). Although the theory has long been abandoned, it is a matter of fact that human brain development has many common processes with development of a rodent brain, particularly in its early stages. This has led to the dominant, simplistic view, that the difference between human and rodent brain is mainly quantitative, i.e. the longer developmental window and larger size of human brain (Fig. 1) account for most differences in the ontogeny (20, 21).

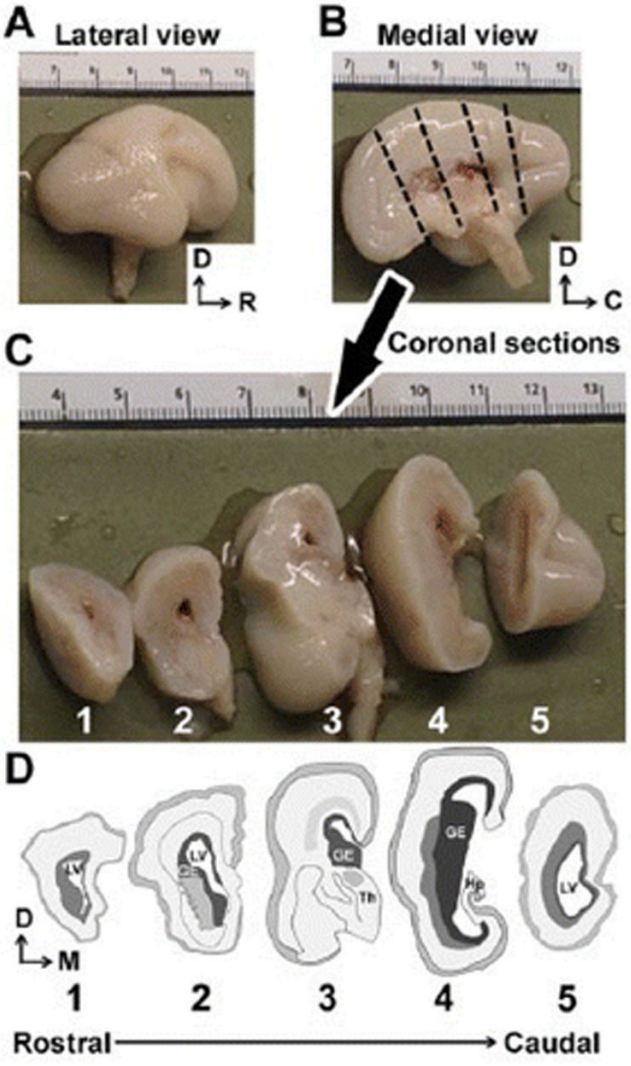

Figure 1. The human fetal brain at mid-gestation. (A) Lateral and (B) medial views of a human fetal right hemisphere at 18 gestational weeks (gw). (C, D) Images and composite drawings of a coronally sectioned right hemisphere, from the rostral (frontal) to the caudal (occipital) pole. D = dorsal; M= medial; C = caudal; R = rostral. Adopted from Ortega et al. (2017), with permission.

Dispute to this time remains unresolved, although many human-specific developmental events have been described. For example, the work in the laboratories of Pasko Rakic and Nada Zecevic in Connecticut has, not long after the discovery of tangential migration of gamma amino butyric acid (GABA)-ergic interneurons from ganglionic eminences to the cortex in rodents (22), shown that in humans the migratory paths of interneurons are more complex (16, 23). In humans, based on the transcription factor expression, at least two lineages of human cortical GABAergic neurons exist. One that expresses DLX1/2 and MASH1, makes up to 65% of the GABAergic interneurons. These cells originate in the ventricular and subventricular zones of the dorsal telencephalon and migrate radially to the cerebral cortex. The other lineage expresses DLX1/2 but not MASH1, and originate in the ganglionic eminence of the ventral telencephalon from where they migrate tangentially across the intermediate zone to the cerebral cortex, similar to what has been reported in rodents (22, 24). Additionally, Rakic and Zecevic (2003) have shown widespread expression of the "ventral" transcription factor NKX2.1 in dorsal cortical regions of human embryo at midgestation (around 20th gestational week). This finding was later corroborated and extended to very early embryonal stages (beginning with 5.5 gestational weeks) (25, 26, 11). It was, however, disputed by several groups since (27, 28, 29), although the evidence is mounting on both sides (30, 31). Time will resolve this dispute, but for the resolution further research on the developing human brain is essential (15, 31). It is fascinating, however, that sometimes very similar results are interpreted by various researchers in quite opposite ways.

ADULT HUMAN BRAIN

Good example for this can be found in two recently published studies in highly respected journals, both from respected scientists. Let us first consider the background: in the sixties Joseph Altman published that new neurons are generated in the adult rat dentate gyrus, which is now known to be crucial for memory (32). This finding was neglected mainly due to skepticism about the brain's capacity for plasticity. In the nineties, with the development of improved techniques for visualizing brain cells, that acceptance of adult neurogenesis became widespread (for a comprehensive review see Gross, 2000), (33). Then, the group of Alvarez-Buylla shook the scientific community by publishing the results of the analysis of many post-mortem human brain samples and discovering that the level of neurogenesis sharply drops already at young age, and is negligible in adults (34). Only a few weeks later, another paper explored the same topics, using the same method – analysis of the post-mortem human brains (35). Most strikingly, both publications describe roughly comparable numbers of newborn neurons in the adult human dentate gyrus, it is only the difference in interpretation. While one group calls the numbers of newborn neurons "negligible" the other considers them "significant". Without going into details of slight differences between the two publications, the main message is that, even with roughly comparable studies, the interpretation of results can be critically important. The

controversy sprouted a wide debate in the scientific community, which by itself was an important and significant achievement (see e.g. Kempermann et al., 2018), (36).

It is noteworthy that examining human brains led to discoveries of important human-specific brain features. For example, a new GABAergic interneuron cell type has recently been discovered in the human neocortex (37). This is congruent with previous work showing great morphological diversity of calretinin-expressing human cortical neurons (38), mirroring thus the more complex development of these neurons (39, 40, 26). Figure 2 illustrates some classical histochemical stainings of the adult human hippocampal formation. It is maybe more fascinating that, even by studying the most evolutionary preserved cortical structures, the archicortex, complexity of neuronal cell types and synapses in the subiculum, for example, exceeds by far what is seen in rodent brains (41, 42). It is, therefore, reasonable to assume that even greater complexity is present in primate-specific and human specific neocortical structures.

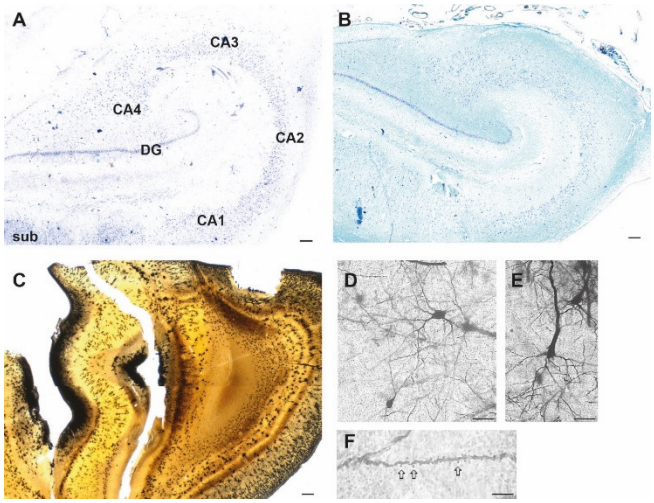

Figure 2. The human hippocampal formation. (A) Nissl staining. Seen are the subiculum (sub), CA1-4 subfields and dentate gyrus (DG). (B) Same area co-stained with Nissl (labels cell nuclei) and Luxol fast blue (staining for myelin). (C) Golgi-Cox staining, random-labeling approximately 1% of all neurons. (D) An interneuron in the CA2 region of the hippocampus stained by Golgi-Cox impregnation. (E) A pyramidal neuron in the CA2 region of the hippocampus. (F) Single dendrite of the pyramidal cell in (E). Arrows point at dendritic spines. Scale bars: 300 µm (A-C); 20 µm (D-E); 5 µm (F).

DISEASED HUMAN BRAIN

It is not only that normal structure of the human brain is a fascinating topic for research, but the diseased brain is even more exciting. It has been self-evident that complex psychiatric disorders such as depression, schizophrenia, autism are human-specific and, although attempts of animal models exist, they are limited to model only certain aspects of disease (43, 44, 45). Furthermore, many neurological disorders, such as multiple sclerosis, Huntington's, Parkinson's and Alzheimer's disease, have not been reported to occur spontaneously in the animal kingdom, and even their animal models fail to reproduce the main symptoms and progression of disease (46, 47, 48, 49). It is thus conceivable that gaining insights into neuropathology of these diseases in brain tissue sections is fundamental for understanding their pathogenesis and designing therapies. For example, demyelinated plaques in multiple sclerosis can be studied in tissue samples from patients (50). Finally, pathological examination of human brain sections was instrumental in discovering the main pathological features of Alzheimer's and Parkinson's diseases, leading to description of diagnostic criteria for these diseases (51, 52). It is a firm belief of the authors that *ex vivo* studies of human brain tissue samples of patients with neurological and psychiatric diseases will play a significant role on our way to find proper causal therapies for these disorders.

PATIENT HM - AN INDEX CASE TURNED INTO ENIGMA

Henry Gustav Molaison (from literature known as "HM") from Manchester, Connecticut was a patient with an intractable epilepsy attributed to a bicycle accident at the age of 7. He developed partial seizures, which after his 16th birthday turned into severe tonic-clonic epilepsy for which he underwent a surgery at the age of 27. William Beecher Scoville, the physician at Hartford hospital who performed surgery on HM's medial temporal lobes claimed and consequently reported that he has excised most of the complete hippocampal formation and adjacent structures, including most of the amygdala and entorhinal cortex (53). HM's seizure subsided, but he subsequently developed severe anterograde amnesia: he could not commit new events to explicit memory. He also had retrograde amnesia, but to a lesser extent and limited to memories not older than 11 years. Interestingly, his ability to form procedural memories was intact: he could learn new motor skills, despite not being able to remember learning them (53). The rest is history of science. Based on this single revelatory case, studies in animal models were prompted to show that the hippocampus plays crucial role in the consolidation of information from short-term to long-term memory (54). Interestingly, further examination of HM's brain done by magnetic resonance imaging (MRI) have hinted that the damage inflicted by surgery to the hippocampus proper was not so severe, whereas the complete entorhinal cortex, subiculum and amygdala were absent (55). Further, after his death (aged 82), HM's brain was serially cut and histologically analyzed, and the authors have confirmed the existence of an almost intact parts of the posterior hippocampus, including DG and CA4 fields (56). Furthermore, Annese et al. (2014) describe the previously unreported damage to the left prefrontal cortex, which could have further implications in HM's memory loss, as this structure has also been considered important for working memory formation in primates and in rodent species it is too small to be properly investigated (as reviewed in Goldman-Rakic, 1996). This brief overview of the HM case offers several insights into human brain research. Firstly, very often discoveries are made due to the

"nature's experiment" – i.e. an unusual accident or disease course in a single patient, which lead to further experiments in animal models, establishing facts about brain function. Second, the true nature of the infliction to human brain can be reviled only posthumously and this, definitive diagnosis is essential to fully understand its impact on behavior.

OUTLOOK

As novel methods of brain imaging, particularly functional imaging emerge, one could falsely assume that old-fashioned morphological analysis of the human brain will be rendered obsolete. This article has tried to demonstrate that there are still many opened questions which are possible to resolve only through diligent analysis of human brain samples at histological and immunohistological level. Among those questions are the gender differences, left-right hemisphere differences, chromosome anomalies (most common being trisomy 21), development of unique structures, expression of adhesion molecules and ion channels. It is fair to say that we have just scratched the surface of possibilities that morphological research of the human brain opens. Therefore, we consider the development of new imaging techniques as complementary, and not substitute for histological research on human brain tissue. At all times, we must consider one of the main questions which drive neuroscience research. The import question, which defines our true nature – if this is our main anatomical difference from animals, then the answer to our uniqueness lies there. If brain is not the only difference, where lies the difference then?

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