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## Review Article

### The Brain as an Energy-integrated Organ: The Relationship between Brain Tissue Structure and Schizophrenia

Masanari ITOKAWA<sup>1,2</sup>, Kenichi OSHIMA<sup>1,2</sup>, Kazuya TORIUMI<sup>1</sup>, Akane YOSHIKAWA<sup>1</sup>, Yasue HORIUCHI<sup>1,2</sup>, Mitsuhiro MIYASHITA<sup>1,2</sup>, Yasuhiro MIYANO<sup>1,2</sup>, Hiroaki ISHIDA<sup>1,2</sup>, Akiko KOBORI<sup>1,2</sup>, Tomoko INOUE<sup>1,2</sup>, Makoto ARAI<sup>1,2</sup>, Youta TORII<sup>3</sup>, Itaru KUSHIMA<sup>3</sup>, Shuji IRITANI<sup>2,3</sup>, Norio OZAKI<sup>3</sup>, Yoshio SUZUKI<sup>4</sup>, Senta NOGUCHI<sup>5</sup>, Rino SAIGA<sup>5</sup>, Ryuta MIZUTANI<sup>5</sup>

1 Tokyo Metropolitan Institute of Medical Science

2 Tokyo Metropolitan Matsuzawa Hospital

3 Nagoya University Graduate School of Medicine

4 High Energy Accelerator Research Organization KEK

5 Department of Applied Biochemistry, Tokai University

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## Abstract

The brain is the major target organ of pathophysiological research on schizophrenia. Compared with other organs, the brain uses a huge amount of energy, equivalent to 20% of the total glucose and 25% of the oxygen consumed in the entire human body, even though it corresponds to only 2% of total body weight. This huge amount of oxygen and glucose is used for producing ATP through mitochondrial oxidative phosphorylation. The vascular system and nervous system closely collaborate to efficiently deliver the energy to the functionally differentiated areas that constitute the whole brain. In relation to this feature of the brain, i. e., glucose oxidation and neurovascular coupling, we review the metabolism and structure of the brains of schizophrenia sufferers. We discuss the extraction of a small subclass with glycation stress, which we propose as a strategy to

resolve the replication issue among schizophrenia studies. We also report schizophrenia and control cases examined using synchrotron radiation nano-CT having high resolution, which allowed us to disassemble the syndrome even to the individual brain level. The nano-CT results indicate that this method can be applied to the investigation of energy metabolism in schizophrenia.

**Keywords:** schizophrenia, capillary, neurovascular coupling, synchrotron radiation

## Introduction

Since Griesinger, W. stated that "psychosis is a brain disease (Geisteskrankheiten sind Gehirnkrankheiten)," neuroscience has been studying the brain as the target organ in the pathology of schizophrenia. According to Nishimaru, Kraepelin, E. placed dementia praecox among metabolic diseases in the fifth edition of his book "Psychiatrie" (1896), along with myxedema, cretinism, and paralytic dementia.<sup>30)</sup> According to Utena, Kraepelin hoped that the neuropathological findings of dementia praecox would eventually be established through the development of natural science.<sup>45)</sup> In this paper, we will first explain the metabolism of glucose oxidation in the brain from the perspective of metabolism and structure, which Kraepelin emphasized, by looking at the mechanism of action potentials in nerve cells, and then we will discuss how the characteristic modular structure of the brain is

functionally supported by the regulation of cerebral blood flow. Furthermore, we will discuss the possibility that the difficulty in reproducing schizophrenia research is due to the heterogeneity of the syndrome, and introduce our research using the large synchrotron radiation facility SPring-8 with the aim of analyzing the structure at the level of individual differences to resolve the problem caused by heterogeneity.

## I. Glucose Oxidation and ATP

Although the brain weight of an adult human is only about 2% of the bodyweight, approximately 16% (54 mL/100 g/min) of cardiac output is used for cerebral blood flow, and approximately 20% (5.4 mg/100 g/min) of the glucose consumed by the whole body and 25% (3.3 mL/100 g/min) of the oxygen supply are consumed by brain activity.<sup>17)</sup> Under physiological conditions, most of this oxygen is used for glucose oxidation (ATP synthesis).<sup>33)</sup>

Using ATP to expel intracellular Na<sup>+</sup> outside the cell, neurons create an ion difference between the inside and outside of the cell (Na<sup>+</sup>: 150 mmol/L outside the cell, 15 mmol/L inside the cell), and maintain an environment with a -60 mV potential difference inside the cell (polarized state) compared with outside.<sup>5)</sup> Only in this environment is it possible to open voltage-dependent Na<sup>+</sup> channels (no energy is required) and allow Na<sup>+</sup> to flow in from outside the cell under the pressure of the concentration gradient, causing the potential to change in a positive direction by 90 mV in the short time of 2 ms (action potential).

Cyclic AMP is also a second messenger for important G-protein receptors that are altered by factors such as dopamine, noradrenaline, serotonin, and acetylcholine, and it is also synthesized from ATP.

From one molecule of glucose, the substrate, two molecules of ATP are produced by glycolysis, and 36 molecules are produced by the TCA cycle. Pyruvate, the final product of glycolysis, changes into lactic acid or ethanol in the absence of oxygen, but enters the TCA cycle in its presence. Therefore, in the brain under hypoxic conditions, glycolysis is promoted by about eight times, but the brain function decreases markedly due to the accumulation of lactic acid. Maintaining

a state of polarization in preparation for action potentials is vital for the brain, and in addition, large amounts of oxygen and glucose are also required for the synthesis of cyclic AMP.

In 1950, Hayashi was the first in the world to discover characteristic changes in glucose oxidation by analyzing the arterial and venous blood of patients with schizophrenia.<sup>12)</sup> In the brain, glucose is broken down into carbon dioxide and water via the TCA cycle. He measured the partial pressures of carbon dioxide and oxygen, as well as others such as lactate, pyruvate, and reduced glutathione, using a total of 377 samples obtained from the internal jugular vein and common carotid artery of a total of 200 patients with schizophrenia and control subjects, who were sampled simultaneously. He classified the partial pressure ratios of carbon dioxide and oxygen into five categories and analyzed them in relation to clinical symptoms, and showed that abnormalities in glucose metabolism and internal respiration are related to the pathology of schizophrenia. It was not until the 1960s that it was discovered that ATP production was due to oxidative phosphorylation in mitochondria, and Hayashi himself did not mention glucose oxidation or ATP, but it was Hayashi's insight that led to the focus on the brain as an energy organ.

In the next section, we will explain how this marked glucose oxidation metabolism in the brain is supported by the vascular system, focusing on the structural characteristics of the brain.

## II. Module Structure and Neurovascular Coupling

Another characteristic of the brain is its modular structure. The brain is a highly functionally differentiated collection of local regions,<sup>33)</sup> and local cerebral blood flow increases so that active modules can efficiently replenish ATP.<sup>34)</sup> The first person to discover this was the Italian physiologist Mosso, A., who measured the volume of the cerebral cortex (oncograph) from the defect in the skull after brain surgery, found that it increased locally in association with mental activity, and concluded that local cerebral circulation fluctuates with psychoneurotic activity<sup>27)</sup> (Figure 1). Ten years after that, Mosso, Roy, C.S. et al. found that stimulating the sciatic nerve of dogs increased blood flow in the somatosensory cortex, and they inferred that the increase in local cerebral blood flow was related to the increase in metabolism caused by neural activity.<sup>35)</sup>

The increase in local blood flow during neural activity is called neurovascular coupling. The structure whereby astrocytes wrap around synapses with their foot processes and

other foot processes of the same cell surround microvessels (Figure 2), is called a neurovascular unit, and this structure suggests that astrocytes are involved in regulation of the caliber of capillaries associated with neural activity. The relationships among neurovascular coupling, glucose oxidation, and ATP production were reported by Leybaert, L. in 2005.<sup>19)</sup> As discussed later, it has been considered that the structure of neurovascular units may affect glucose oxidation, etc., based on signal attenuation models such as the cable theory and fluid dynamics such as Reynolds stress.

Since 1974, when Ingvar, D.H. et al. reported a decrease in blood flow to the frontal region (hypofrontality) in schizophrenic patients at rest,<sup>13)</sup> there have been reports of a decrease in neurovascular coupling in various situations, including during task performance. According to a meta-analysis of 19 studies (557 schizophrenia patients and 584 controls) of FDG-PET using perfusion weighted images of cerebral blood flow and radioactively labeled fluorodeoxyglucose (FDG), differences in coupling between glucose metabolism and cerebral blood flow were observed between patients and controls in 10 regions, including the anterior insula, dorsal anterior cingulate cortex, putamen, and temporal pole.<sup>40)</sup> However,

there are also neuroimaging studies that dispute the reproducibility of hypofrontality<sup>22)</sup> and reports that even question its existence.<sup>46)</sup> This is a reproducibility problem that is characteristic of schizophrenia research, which has also been seen in genetic research and clinical trials of therapeutic drugs ("Schizophrenia is the graveyard of neuropathologists." Plum, 1972). This reproducibility problem was proposed by Kraepelin, who emphasized the longitudinal course of schizophrenia, as a type of "chronic progressive" disease, and therefore it is possible that it does not have the same homogeneity or clear boundaries as natural species; in other words, it may contain heterogeneity. In order to resolve this heterogeneity, we considered it necessary to differentiate the subjects recruited using the operational diagnostic criteria into subtypes smaller than the disease unit, and then reconstruct them into a homogeneous set. If we take this strategy to its extreme, this will result in a subdivision down to the level of individual differences. In the next section, we will introduce research at the cellular level for smaller subtypes.

### III. Individuality of Cell Shape

Individual differences at the cellular or tissue level, not just in brain neurons, have been studied in the fields of

pathology and histology. For example, tumor tissue shows different structures in different cases, and this histological diversity of tumors is considered to be derived from individual differences at the genetic level.<sup>7)</sup> There are also reports that the heterogeneous microenvironment in which tumor cells are located affects the prognosis, and that this is related to the genetic mutations in each case.<sup>29)</sup> Individual differences in biological structures, not just in tumor tissue, have long been discussed in relation to cell division,<sup>4)</sup> but research into the individuality of cell shapes themselves has not progressed. This is thought to be because it is difficult to distinguish between intrinsic biological properties and exogenous physical and chemical factors, just as the shape of cultured cells is affected by the medium environment. Another reason is that, even though cells are essentially three-dimensional, under a microscope they can only be observed two-dimensionally from the direction determined by the observer, and there is also anisotropy in resolution that depends on the viewing direction.<sup>38)</sup> In the next section, we will discuss how the morphology of neurons in schizophrenia has been examined, and in the following section, we will introduce our own research, in which we have investigated individual differences in neuronal morphology.

#### IV. Neuropathology of Schizophrenia

Even with the limitations mentioned above, research has been carried out from various perspectives on structural changes in neurons caused by mental disorders. In 1960, Tatetsu examined brain tissue sections from patients with schizophrenia in a comprehensive manner and reported on several characteristics, including the meandering of neuronal processes, which he had also observed in the aforementioned case study.<sup>43)</sup> Furthermore, he conducted ultrastructural analysis using electron microscope images, and noted marked development of the Golgi body in nerve cells and the accumulation of a peculiar structure at the boundary between oligodendrocytes and axons, and pointed out the possibility that the metabolism of nerve cells was inhibited due to some kind of enzyme dysfunction.<sup>23)</sup> In addition, research has been conducted on the morphology of neurons in schizophrenia cases, particularly in the hippocampus, and it has been reported that the size of cell bodies differs between schizophrenia patients and control cases<sup>50)</sup> and that the orientation of pyramidal cells differs significantly.<sup>18)</sup> However, these results are difficult to reproduce in some cases, and presently it is difficult to say that

there is a consensus regarding the neuropathology of schizophrenia.<sup>10)</sup>

From a macroscopic perspective using CT and MRI, there has been some progress in understanding cerebral ventricle enlargement in schizophrenia<sup>15)21)</sup> and atrophy of specific areas of the brain.<sup>16)39)42)</sup> While these macroscopic brain changes have been reported, the reason why there has been no accumulation of knowledge about cells and blood vessels that make up brain tissue may be because schizophrenia is an extensive syndrome with heterogeneity, and because brain tissue, which exists in three dimensions, has been observed under a microscope using thin sections cut from the tissue and viewed from only one direction. One way to image brain tissue in three dimensions from all directions is to use CT, but unfortunately this has a resolution of only a few millimeters, so it is not possible to see cells or nerve processes. However, it is possible with synchrotron radiation micro-CT and nano-CT methods, shown in Figure 3.<sup>24)</sup> With these methods, we can even visualize intracellular organelles by performing CT with a resolution 1,000 to 10,000 times greater than normal. A resolution of 10,000 times in a two-dimensional image means that the brightness of the X-rays must be 100 million times brighter. This requires special measurement facilities, such as

the SPring-8 large synchrotron radiation facility (Hyogo Prefecture) and the Advanced Photon Source (Illinois, USA).

As I mentioned at the beginning of this article, when we consider the brain as the central organ in the pathology of schizophrenia, it is a metabolic organ that carries out marked glucose oxidation, and it is an organ that is made up of a collection of specialized parts that are not present in other organs, such as modular structures, so neurovascular coupling is what guarantees the normal operation of the brain with energy inflation. As mentioned earlier, in order to tackle the issue of reproducibility in schizophrenia research, which has been a problem for over 100 years, it is necessary to analyze blood vessels and nerves with a resolution down to the level of individual differences, and SPring-8 is an analysis device with the ability to do this.

## V. Structural Characteristics of Brain Tissue

We have been conducting research to analyze the three-dimensional structure of autopsied brain tissue from patients with schizophrenia and matched control subjects of the same age and sex, using synchrotron radiation micro-CT and nano-CT methods.<sup>14)25)26)36)</sup> We handle brain

tissue samples with the utmost care to protect privacy, after obtaining written consent from the bereaved families. The use of human tissue in research was approved by the Ethics Committee for Research on Human Subjects at Tokai University, the Clinical Research Review Committee of the Faculty of Medicine at Tokai University, the Ethics Committee of the Tokyo Metropolitan Institute of Medical Science, and the Institutional Biosafety Committee of Argonne National Laboratory in the United States, and the research was conducted in accordance with the approved conditions. The areas of the brain studied were the anterior cingulate gyrus (Brodmann area 24, BA24) and superior temporal gyrus (BA22), both of which have been reported to show volumetric changes in schizophrenia.<sup>8)31)48)</sup> Brain tissue was stained using the Golgi method, and then embedded in epoxy resin in borosilicate glass capillaries to form the measurement samples. In the Golgi method, mainly neurons and blood vessels are stained by silver and visualized as X-ray images. The structures depicted in these three-dimensional images are traced, the neural and blood vessel networks are reproduced in a Cartesian coordinate system, and the characteristics of the structures are examined using differential geometry based on the

coordinate values. Figure 4 shows tracing of the blood vessel network. In the capillaries, blood cells can be seen crowding the lumen.<sup>36)</sup>

The same analysis was performed involving a total of 89 structures in the neural network, and the statistical parameters of the structures were obtained in four schizophrenia patients and four controls.<sup>25)26)</sup> Figure 5 shows the results for one patient. Here, the curvature of the nerve processes and thickness (radius) of the dendritic spines that form synapses are plotted for each dataset. Although these structures are treated separately in neuroscience, the mean and standard deviation of both structure parameters are similar within patients, but the values differ between patients, and the plots are discontinuous and stair-shaped. In other words, these structure parameters suggest the possibility that they exhibit unique values in each patient.

When we examined the structural parameters of neurites and capillaries, we identified a correlation between them. As can be noted in Figure 6, we examined the relationship between the curvature and thickness of neurites and capillaries based on a total of 24 structures.<sup>36)</sup> In both schizophrenia patients and controls, there was a significant correlation between the curvature of neurites and capillaries

(Figure 6a), supporting the possibility of a structural relationship between neurons and blood vessels. Neurons are ectodermal cells that differentiate from the neural plate, while blood vessels are formed mainly from the mesoderm, along with bones and muscles. The fact that the structure of cells that split early in development shows a correlation suggests that curvature may be a fundamental property that is determined at the undifferentiated stage before the gastrula. However, the thickness of capillaries is constant regardless of the thickness of neurites (Figure 6b), and the thickness of capillaries may be determined by non-fundamental properties - physical constraints such as the passage of blood cells through blood vessels - rather than curvature conditions.

## VI. Neurites and Capillaries in Schizophrenia

The curvature of neurites determined in this way was significantly greater in patients with schizophrenia than in controls (Figure 7a). In general, thin wires bend sharply, while thick wires bend only gently. The same is true for neurites, and their curvature was inversely proportional to their thickness (Figure 7b). Therefore, in schizophrenia, marked curvature is equivalent to thin neurites, and the variation between brain regions tends to be greater than in

controls (Figure 7a). Thin neurites are unfavorable for the transmission of action potentials,<sup>9)</sup> and the brain tissue may be functionally impaired.

In addition, the high-level curvature of neurites in schizophrenia patients means that the capillaries are also curved and meandering, as shown in the correlation in Figure 6a. However, Figure 6b shows that the thickness of capillaries is constant. Thus, blood vessels that have a constant thickness and meander have a larger volume ratio in tissue than straight blood vessels, and this can affect the balance between blood flow and metabolism. In addition, when fluid meanders, the Reynolds stress increases viscosity and causes a decrease in the flow rate,<sup>47)</sup> suggesting that blood flow may be lower in the brains of people with schizophrenia than in controls. Changes in cerebral blood flow<sup>2)20)32)</sup> and metabolism<sup>3)40)</sup> in schizophrenia patients have been reported, and it is possible that these structural characteristics of capillaries are involved.

The greater the curvature of the nerve projection, the thinner the fiber. The action potential transmitted along nerve fibers can be explained by the physical model of ion exchange between the inside and outside of the axon through the cell membrane (cable theory).<sup>6)</sup> In this model, the transmission of action potentials is

described by likening nerve fibers to cables. According to this, the distance between two points connected by a nerve fiber becomes longer as the curvature becomes more marked, and the fiber becomes thinner, so the action potential is attenuated. In other words, this suggests that in schizophrenia patients, the connections with neurons that are further away than normal are impaired.

The results of analysis of the structure of cerebral blood vessels and nerve processes using SPring-8 suggest that the brains of people with schizophrenia show structural changes that promote energy inflation. These results show that synchrotron radiation nano-CT is useful not only for elucidating the structure of brain tissue, but also for helping us to understand the energy metabolism of the brains of people with schizophrenia.

Our research is the result of 10 years of analysis of over 10,000 nerve processes and blood vessels over 1 M in length. The analysis was carried out blindly, without knowledge of the cases, or setting any preliminary working hypotheses, and the data are from brain tissue, examining structural parameters as they are, using all the results obtained. Although we need to accumulate more cases in the future, the data so far indicate that there is individuality in the structure of brain

tissue, and that this changes significantly in the presence of schizophrenia. These studies were announced in a press release by Argonne National Laboratory in the United States,<sup>37)</sup> and have already attracted a variety of responses. The macroscopic changes in brain tissue of schizophrenia patients<sup>8)15)16)21)31)39)42)48)</sup> are widely recognized, and we believe that one of the reasons for this involves the structural changes in nerve processes and capillaries in brain tissue.<sup>25)26)36)</sup>

### Conclusion

The human body is a biochemical system that is supported by the transport of substances through the vascular system, and life functions are maintained by the exchange of substances and energy consumption through capillaries. Therefore, in organs that consume a lot of energy, such as the brain, the structure of the capillaries is "built" accordingly. However, there are still many unknowns regarding the three-dimensional structure of the capillaries in the brain. In the above-mentioned case study, the structure of capillaries in the cerebral cortex was examined based on geometric parameters such as curvature, and this is the first report of such an examination, including other tissues and organs, and the first time

that a correlation with cell structure has been examined. This kind of research has become possible due to synchrotron radiation CT, which can faithfully reproduce three-dimensional structures.<sup>14)</sup> It is considered necessary to apply this method to other mental and neurological disorders and investigate the kinds of geometric properties the capillaries exhibit. It would also be interesting to see what differences emerge when comparing the brain with other tissues and organs that have low energy consumption.

Analysis of the brain's ultrastructure using synchrotron radiation CT has revealed a structural correlation between capillaries and nerve processes. This can be rephrased as finding a relationship between energy supply and information processing within the structure of brain tissue. In other words, it means that pathological information processing can affect the structure of capillaries, and in schizophrenia, this may be a constant progression. At present, analysis using synchrotron radiation CT is limited to the four schizophrenia patients and four controls described above, so it is urgent that we increase the number of cases to enrich our findings. In addition, it is also necessary to investigate the biochemical mechanisms that connect the two structures, and determine what changes occur in schizophrenia in the

process that links energy supply to glucose oxidation and then to neural activity.

As a possible mechanism, we would like to present one hypothesis. Reactive oxygen species produced during the process of glucose oxidation in mitochondria cause non-physiological glycation modification - glycation stress. We examined patients with schizophrenia who did not have kidney dysfunction or diabetes (the two main causes of glycation) and controls, and found that 40% of the patients with schizophrenia also showed signs of glycation stress, including cases where the DNA region encoding the mitochondrial regulatory molecule (microRNA with frataxin regulatory ability) was deleted.<sup>49)</sup> In order to rule out the effects of medication, we analyzed the brains of mice in which glycation stress had been reproduced genetically and environmentally, and found that the expression of genes related to mitochondria had changed most markedly.<sup>44)</sup> Such glycation stress may alter the shape of blood vessels and nerve cells through its effects on cellular activity, and could be one of the mechanisms determining the structural correlation between blood vessels and nerve cells.

The results described above support the changes in metabolism and

structure that Kraepelin emphasized in the pathology of schizophrenia.

#### Note

One of the authors, Mizutani, was conducting measurement experiments on human brain tissue at the SPring-8 large synchrotron radiation facility at the beginning of June, before the start of the rainy season in Japan. Generally, this type of facility operates 24 hours a day, so experiments are carried out in shifts around the clock. Mizutani was in charge of the morning shift, from 1 AM to 1 PM. After a week of this, he was handing over to the afternoon team and chatting about the neurons he had seen that day (Fig. 3). The team taking over said, "That's the same as patient A, who we measured before." In fact, the sample from that morning was indeed patient A, and additional measurements were being taken to supplement the amount of data. Patient A had structural characteristics that could be easily verbalized, and was one case where individual identification was possible simply by talking about them in a conversation. In this way, the three-dimensional structure of nerve cells was determined as showing characteristics that are unique to each individual, and if seen by someone who knows what to look for, they can be used to identify individuals, just like a face.

### Conflict of Interest

Itokawa and Arai hold a patent for the application of pyridoxamine as a treatment for schizophrenia. Ozaki has the following COI relationships with companies:

Scholarship donations: Otsuka Pharmaceutical Co., Ltd., KAITEKI Institute, Inc., Dainippon Sumitomo Pharma Co., Ltd., Eisai Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Meiji Seika Pharma Co., Ltd., and Astellas Pharma Inc.

Honoraria for lectures: Otsuka Pharmaceutical Co., Ltd., Eisai Co., Ltd., Takeda Pharmaceutical Company Limited, Pfizer Japan Inc., Janssen Pharmaceutical K.K., Meiji Seika Pharma Co., Ltd., Astellas Pharma Inc., and MSD K.K.

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2019A1207, 2019B10 87, 2020A0614, 2020A1163, 2021A1175. Synchrotron radiation experiments at the Advanced Photon Source were performed under GUP-45781 and GUP-5 9766. This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

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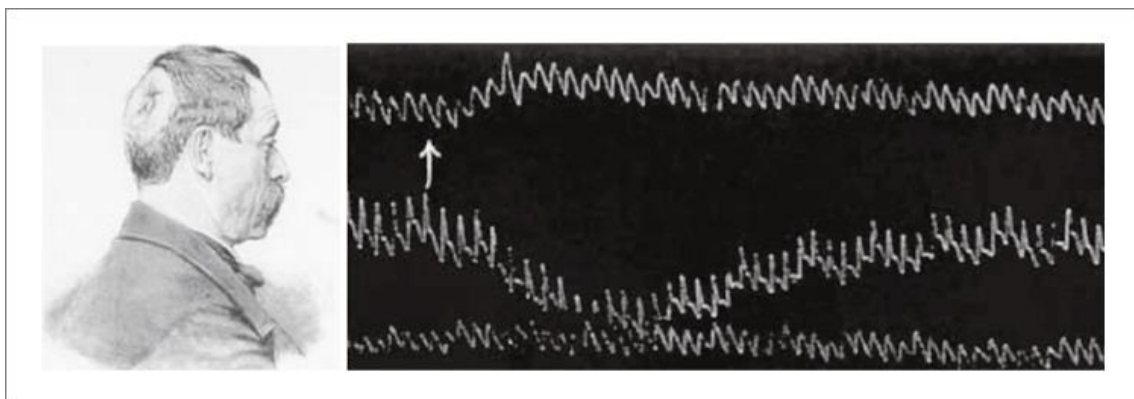


図1 神経活動と脳血流

症例 (Cane, L.) の頭蓋骨欠損部 (左)。刺激時の足容量変化 (右上波形), 刺激時の脳容量変化 (右中波形), 安静時 (右下波形)。情動刺激「Cane 氏が妻に初めて会ったときの印象を実験者が尋ねた」を与えたとき (右矢印)。(文献 27 より引用)

Figure 1: Neural Activity and Cerebral Blood Flow

A skull defect (left) in a case (Cane, L.). Changes in foot capacity during stimulation (upper right waveform), changes in brain capacity during stimulation (middle right waveform), and at rest (lower right waveform). When the emotional stimulus: "the experimenter asked Mr. Cane about his first impression of his wife," was given (right arrow). (Adapted from reference 27)

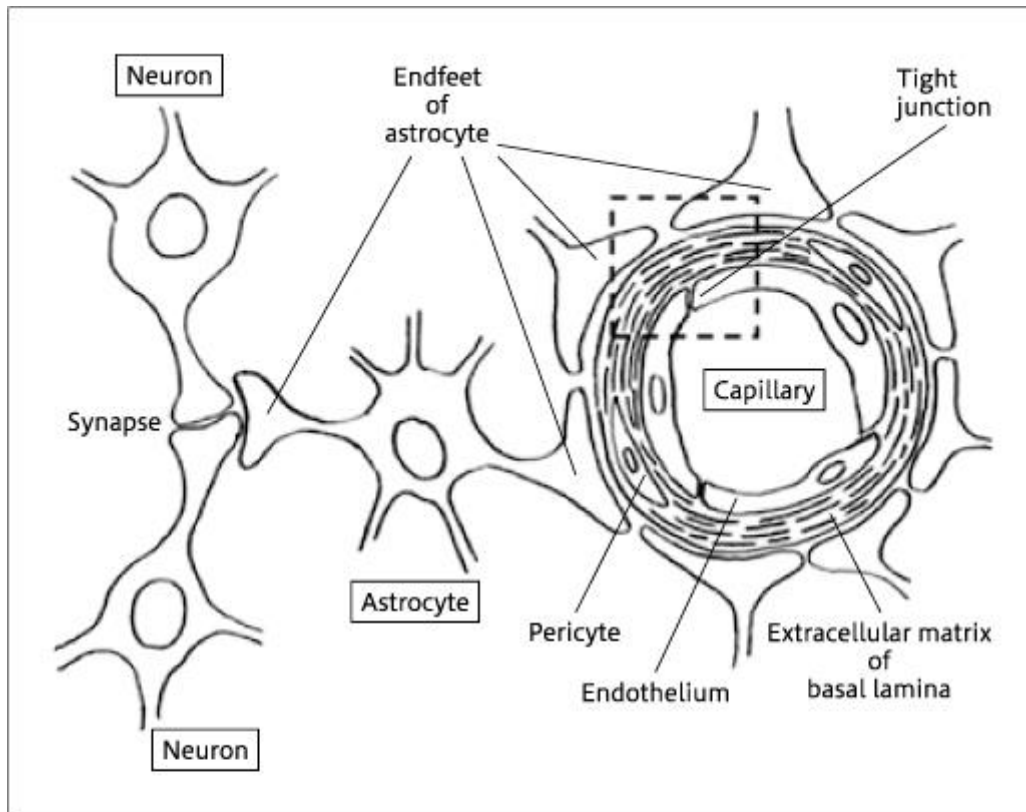


図2 神経血管ユニット

神経細胞と血管をアストロサイトの足突起がつなぎ、神経血管カップリングを支えている。血管を包み込んだアストロサイトの足突起は、周皮細胞（ペリサイト）、内皮細胞、基底膜と連携して血液脳関門を形成する。統合失調症では血液脳関門の透過性亢進が報告され、脳の免疫特権の障害が示唆されている<sup>28)</sup>。最近、Haruwaka, K. らはリポポリサッカライドをマウスに投与した全身炎症モデルを用いて、クローディン5 (CLDN5) を介してアストロサイトの足突起をミクログリアが貪食していることを見だし、血液脳関門の破綻を示唆した<sup>11)</sup>。有岡らは、染色体22q11.2欠損患者の染色体欠失領域にCLDN5がコードされていることを指摘し、同欠損患者の10~30%で統合失調症が併発することから、統合失調症の病態に脳血液関門の機能低下がかかわる可能性を述べている<sup>1)</sup>。(図は文献41より引用)

Figure 2: Neurovascular Unit

Astrocyte foot processes connect neurons and blood vessels, supporting neurovascular coupling. Astrocyte foot processes that wrap around blood vessels work in conjunction with pericytes, endothelial cells, and the basal membrane to form the blood-brain barrier. In schizophrenia, increased permeability of the blood-brain barrier has been reported, suggesting impairment of the brain's immune function.<sup>28)</sup> Recently, Haruwaka, K. et al. used a systemic inflammation model in which lipopolysaccharide was administered to mice, and found that microglia phagocytose astrocyte foot processes via claudin 5 (CLDN5), suggesting that the blood-brain barrier is disrupted.<sup>11)</sup> Arioka et al. pointed out that CLDN5 is encoded

in the chromosomal deletion region of patients with chromosome 22q11.2 deletion, and since 10-30% of patients with this deletion also suffer from schizophrenia, they suggested that a decrease in the function of the blood-brain barrier may be involved in the pathology of schizophrenia.<sup>1)</sup> (Adapted from reference 41)



**図3 大型放射光施設 SPring-8 での測定の様子**

後ろの「37XU」と書かれた扉を入ると測定装置があり、ヒト脳組織がセットされている。X線を遮蔽する重厚な扉の向こうでは、写真中央奥から強力なX線ビームが手前に向かってきて脳組織を透過し、対物レンズなどを通して、右後方約25m先のカメラでX線像が撮像されている、巨大な測定設備である。写真では、座っている著者の1人の水谷が左手のスイッチで脳組織の位置を調整している（注）。

Figure 3: Measurement at the SPring-8 Large Synchrotron Radiation Facility

If you go through the door marked "37XU" at the back, you will find the measurement device, with a human brain tissue set up inside. Behind the heavy door that shields from the X-rays, a powerful X-ray beam comes from the center of the back of the photo and penetrates the brain tissue, passing through the objective lens and other equipment to be imaged by a camera about 25 m to the rear on the right, in this huge measurement facility. In the photo, Mizutani, one of the authors sitting down, is adjusting the position of the brain tissue with the switch on the left (Note).

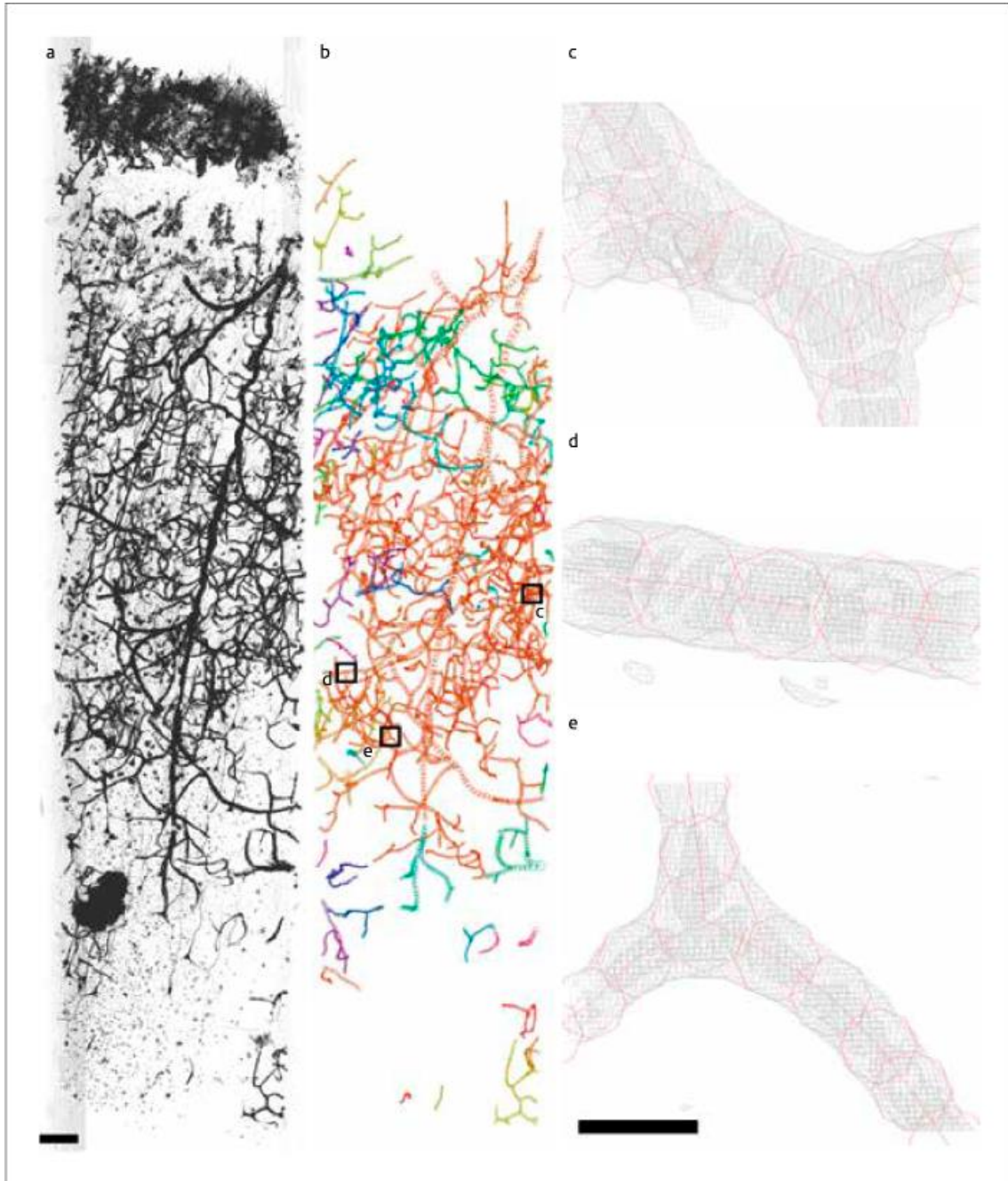


図4 統合失調症例の側頭葉BA22野の脳組織の構造 (a) と、その血管ネットワークをトレースしてデカルト座標系で再現したモデル (b)

a : 上端が脳表、スケールバー : 100  $\mu\text{m}$ 、

b : 黒い四角で示した部分を、c~e で拡大して示した、

c~e : 灰色は三次元像で、毛細血管内腔に血球が観察される。赤の八角形は血管をトレースしたモデル、スケールバー : 10  $\mu\text{m}$ 、

(文献 36 より引用)

Figure 4: Structure of Brain Tissue in the BA22 Area of the Temporal Lobe in a Schizophrenia Patient (a) and a Model Reproduced in a Cartesian Coordinate System by Tracing the Vascular Network (b).

a: The top edge is the surface of the brain. Scale bar: 100  $\mu\text{m}$ .

b: The area indicated by the black square is enlarged in c-e.

c-e: The gray area is a three-dimensional image, and blood cells can be seen in the lumen of the capillaries. The red octagons are the traced model of the blood vessels.

Scale bar: 10  $\mu\text{m}$ .

(Adapted from reference 36)

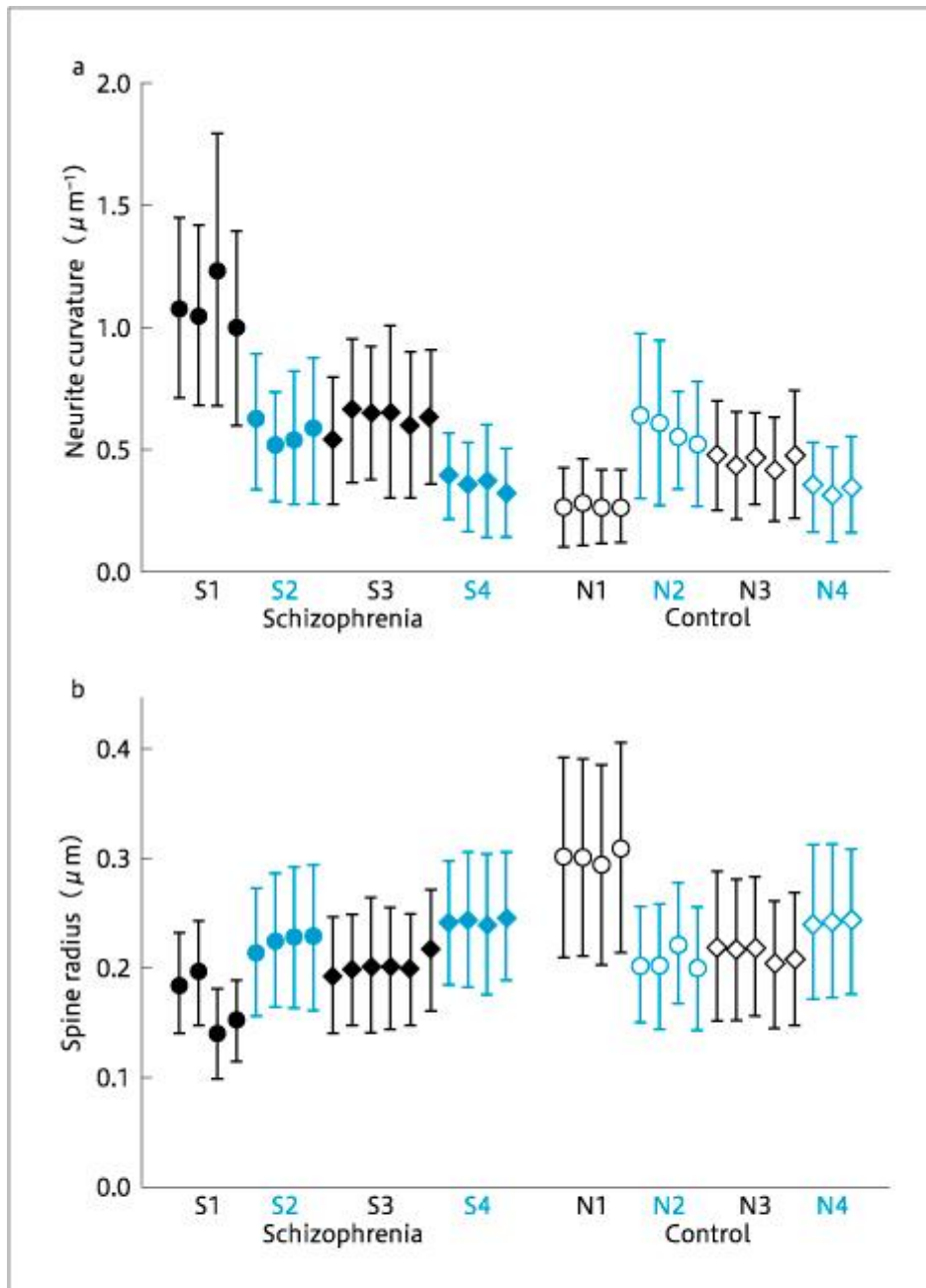


図5 側頭葉 BA22 野の神経突起の曲率 (curvature) (a) と、棘突起 (dendritic spine) の半径 (b) を、データセットごとにプロットした

統合失調症例 S1~4 と、対照例 N1~4 を記号等で区別している。中央のマークが平均値、バーが標準偏差を表す。どちらの構造パラメータも、症例内ではおおむね一定の値を示すが、症例間では値が異なり (神経突起の曲率  $P=2.9 \times 10^{-8}$ ; 棘突起の半径  $P=4.5 \times 10^{-8}$ ; Welch's ANOVA), stepwise なプロットとなる。(文献 26 より引用)

Figure 5: Curvature (a) and Radius (b) of Dendritic Spines in the BA22 Region of the Temporal Lobe Were Plotted for Each Dataset.

Schizophrenia patients S1-4 and controls N1-4 are distinguished by symbols. The central mark indicates the mean value, and the bar indicates the standard deviation. Both structural parameters show generally consistent values within each case, but differ among them (curvature of dendrites  $P=2.9 \times 10^{-8}$ ; radius of dendrites  $P=4.5 \times 10^{-8}$ ; Welch's ANOVA), resulting in a stepwise plot. (Adapted from reference 26)

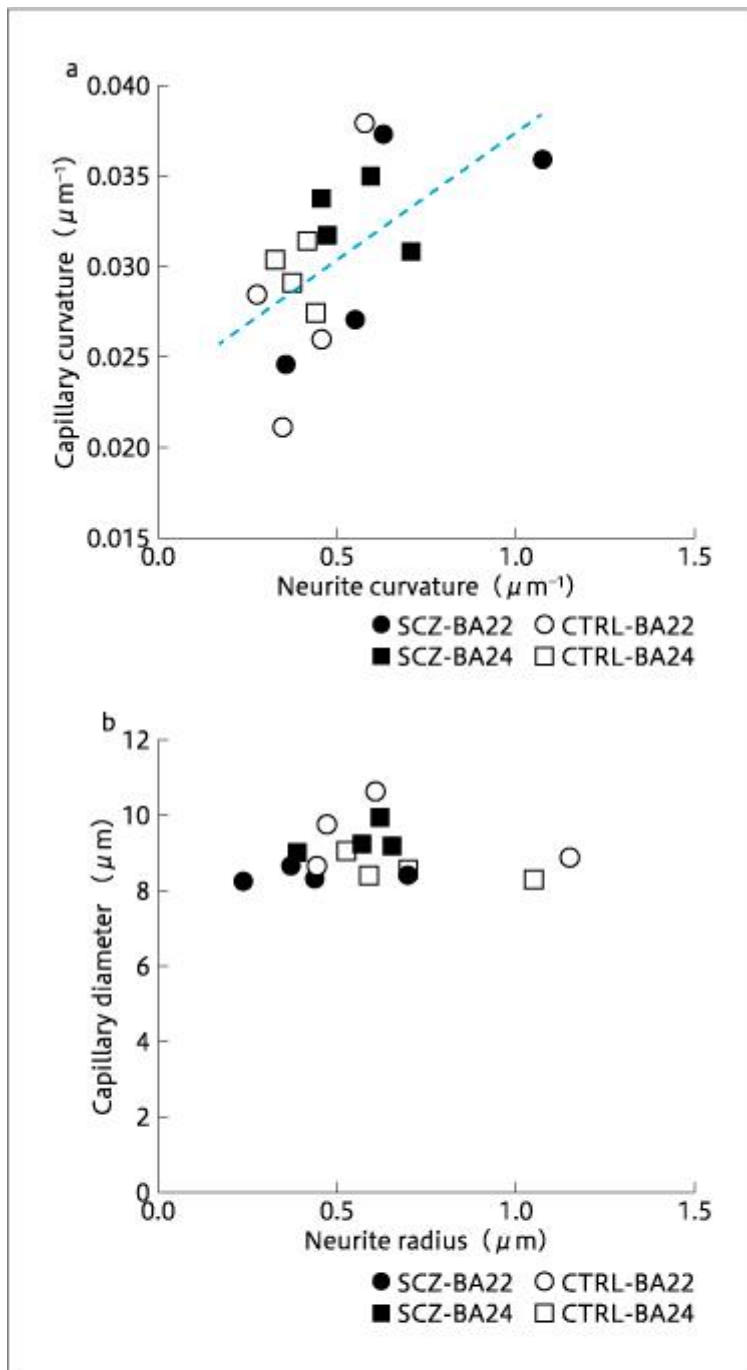


図6 毛細血管（縦軸）と神経突起（横軸）の関係

- a : 毛細血管と神経突起の曲がり方（曲率（curvature））に有意な相関がみられる（Spearman's  $\rho=0.63$ ,  $P=0.011$ ）。破線で直線回帰を示す。
- b : 毛細血管と神経突起の直径あるいは半径をプロットした。毛細血管は一定の直径を示し、神経突起の太さとは関係しない。

（文献 36 より引用）

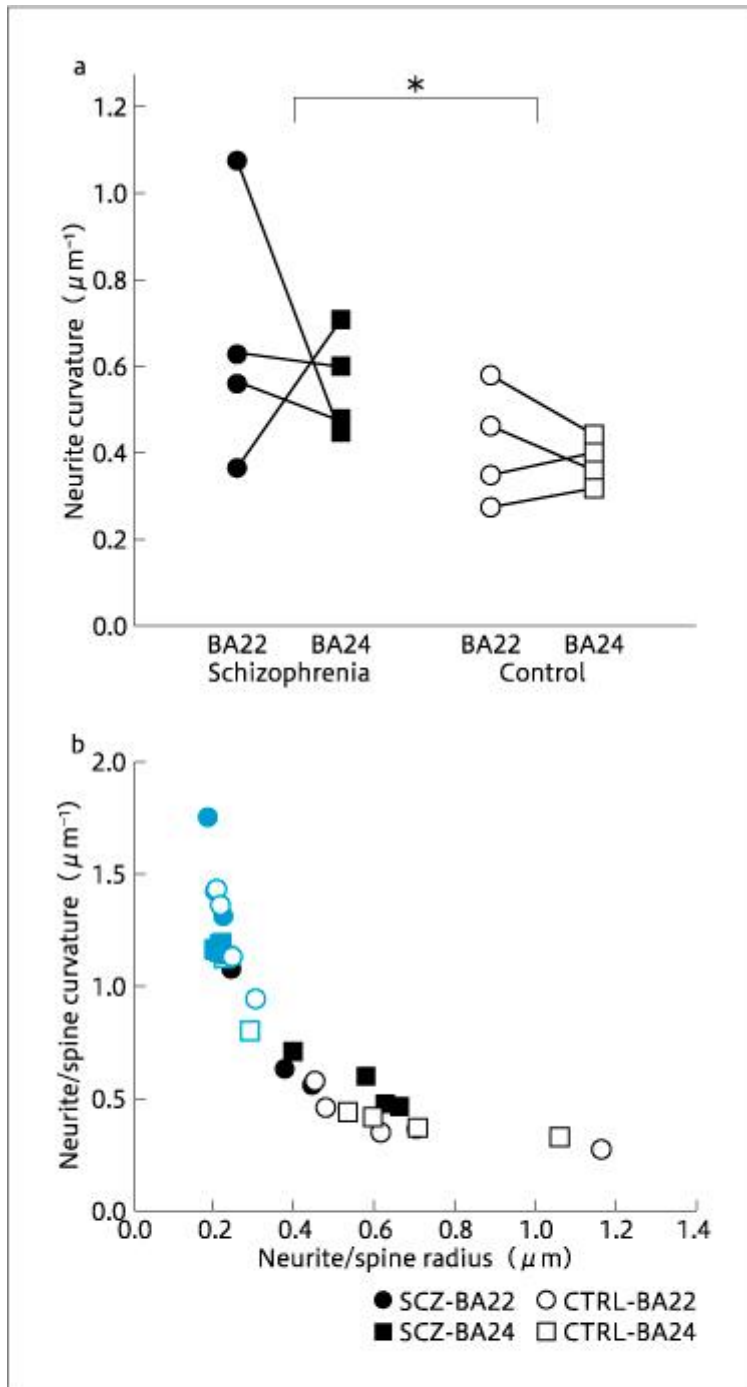
Figure 6: Relationship Between Capillaries (vertical axis) and Neurites (horizontal

axis)

a: There was a significant correlation between the curvature of capillaries and neurites (Spearman's  $\rho = 0.63$ ,  $P = 0.011$ ). The dashed line shows a linear regression.

b: The diameter or radius of the capillaries and nerve processes was plotted. The capillaries showed a constant diameter and were not related to the thickness of the nerve processes.

(Adapted from reference 36)



**図7 神経突起・棘突起の構造パラメータ**

a : 統合失調症と対照例の神経突起の曲率 (curvature)。統合失調症では、値が有意に大きく (\* $P=0.031$ , two-way ANOVA, 疾患/対照と脳部位を2因子とした), 部位間でばらついている。

b : 曲率と太さの反比例関係。神経突起を黒で、棘突起を青色で示した。

(文献 26 より引用)

Figure 7: Structural Parameters of Nerve Processes and Spines

a: Curvature of nerve processes in schizophrenia patients and controls. In schizophrenia patients, the values were significantly larger ( $P=0.031$ , two-way ANOVA, with disease/control and brain region as two factors), with variation between regions.

b: The inverse relationship between curvature and thickness. Neurites are shown in black, and spines are shown in blue.

(Adapted from reference 26)